OESTRUS INDUCTION IN PRIMIPAROUS LACTATING RABBITS BY A 48 HOURS MOTHER-LITTER SEPARATION: ENDOCRINE AND BEHAVIOURAL RESPONSES

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ABSTRACT

Primiparous does express low mating acceptance during lactation and different oestrus induction methods are employed to improve sexual receptivity. The aim of this work was to study the effect of a biostimulation, a 48 hours doe-litter separation (DLS), in primiparous lactating rabbits on: 1) mating acceptance at day 11 and days 12-14 *postpartum* ; 2) external characteristics of the vulva; 3) plasma concentration of estradiol (E2), testosterone (T), progesterone (P4) and cortisol (CT). Seventy primiparous does from local population were divided at day 1 after kindling, in two equal groups: control (C, n=35) and DLS (n=35). Litter size was equalised to 6-7 kits. The does had free access to the nest-box, except from day 9 to day 11 *postpartum* (PP) for the DLS group. For doe-litter separation, the nest-box entry was closed. Blood samples were obtained from 10 does per group at days 9 and 11 PP, before mating. The results showed that the DLS treatment improved the mating acceptance at day 11 PP (88.5 *vs.* 54.2%, P<0.005) and the frequency of red or pink and turgid vulva (54.3 *vs.* 20%, P<0.005). DLS does had the lowest percentage of white vulva (20 *vs.* 48.6%, P<0.05). The sexual receptivity of DLS does depended on the time for which the female was presented to the male: 60 % refused mating before opening the nest-box, while 80.9% of them accepted the male after the nursing episode. From day 12 to day 14 PP, the sexual receptivity was higher in DLS group than in the control group (75 *vs.* 43.7%), the difference was however not significant (P>0.05). Estradiol, Testosterone and Progesterone plasma levels in DLS group rose significantly from day 9 to day 11 PP (+44.4%, +59.2% and +82%, respectively, P<0.01) compared to control group (+6.7%, -3.9% and +33.4%, respectively, P>0.05). In contrast, cortisol plasma levels were not affected by the DLS treatment (-12.4% for control, P>0.05, and -14.4 % for DLS group, P>0.05). The percentage of "high progesterone syndrome" (P4>1ng/ml) was not different between groups (30 *vs.* 40%, for DLS and C respectively, P>0.05). The 48h doe-litter separation acted as an efficacy oestrus inductor in primiparous lactating rabbits. This biostimulation method induced modifications to the steroidogenic activity of the ovary, particularly by enhancing the secretion of estrogens and testosterone, and consequently, by influencing positively the sexual behaviour of the does.

Key words: Reproduction, biostimulation, rabbit does, sexual receptivity, steroid hormone.

INTRODUCTION

The 48 hours doe-litter separation (DLS) at day 9 after kindling improves the sexual receptivity (SR) and consequently, fertility of the lactating does, with the same efficiency as eCG (Maertens *et al.,* 1998). The physiological mechanisms by which this method improves the sexual receptivity are not clearly definite, and particularly the endocrine response. The transient separation enhances number of follicles greater than 1 mm (Rebollar *et al.,* 2008), plasma concentration of estrogens (Ubilla *et al.,* 2000a), and FSH and LH (Cano *et al.*, 2005). The study of the influence of DLS on prolactin blood level leads to contradictory results (Ubilla *et al.* 2000b; Rebollar *et al.* 2006; Cano *et al.*, 2005). Despite sexual receptivity in rabbit is influenced by androgen (Beyer and Mc Donald, 1973), the effect

of 48 h DLS on testosterone secretion has not been yet explored. No study has also investigated the effect of DLS on adrenal activity, since temporary separation of the mother from her litter could determine social stress in the doe. On the other side, the effect of a 48 hours doe-litter separation on sexual receptivity was rarely experimented in natural mating and the majority of the works scored the sexual receptivity on the basis of the external characteristics of the vulva. The International Rabbit Reproduction Group (IRRG) considered however the mating acceptance as the best method for checking the sexual receptivity (IRRG, 2005).

The aim of this work was to evaluate the effect of a 48 hours doe-litter separation on estradiol, testosterone, progesterone, and cortisol plasma levels, and on sexual receptivity in primiparous lactating does submitted to natural mating.

MATERIALS AND METHODS

Animals and experimental design

The trial was conducted in the experimental rabbitry of the National Veterinary School of Algiers. A total of 70 nulliparous does and 18 bucks from the local rabbit population were used. The animals were housed in a naturally ventilated building, maintained under natural light-dark photoperiod. The mean ambient temperature varied from 17 to 22°C, and the relative hygrometry from 62 to 77%. The rabbits were fed *ad libitum* with a commercial pellet diet and have free access to water, with nipple drinkers. The does were reared into individual flat-deck cages in wire mesh (46.5cm x 62cm x 29cm height), with a closable nest-box in wood (43cm x 26cm x26 cm height), hanging out of the mother cage. The nulliparous does were first mated at the age of 4.5 to 5 months, with a mean weight of 3.1± 0.2 kg. At day 1 after kindling, the litter sizes were equalised to 6-7 kits, and the does were randomly allotted to one of the two experimental groups, with 35 females in each group:
- Control group (C): the does have always free access to the nest box.
- Doe–litter separation group (DLS): Free suckling was adopted, except during the 48 h doe-litter separation. The nest-box entry was closed from 11:00 a.m. of day 9 PP to 11:00 a.m. of day 11 PP.

At day 11 PP, all does were presented to a maximum of three males, until they accept the mating. To test the influence of suckling on sexual behaviour, the DLS does were presented to a maximum of two males before the nursing, and, if not receptive, again to another immediately after nursing. All non receptive does of both groups were presented again at days 12, 13, and 14 PP until they accepted the mating. Before the presentation to the first buck, the colour and turgidity of the vulva were checked by visual evaluation. Four colours were attributed (red, pink, purple and white) and two levels for turgidity (yes or no), accordingly to the recommendations of the IRRG (2005).

Blood sample collection and hormone assays

Blood sample (4 ml) was collected from the marginal ear vein into heparinised tube and immediately centrifuged at 1000 x g for 10 min. Plasma was aliquoted and stored at -20 °C until assay. Blood samples were obtained from 10 does per group at days 9 and 11 PP, between 10:00 and 11:00 a.m., before presentation to the male, and for separated does, before opening the nest-box. Plasma estradiol, progesterone, testosterone and cortisol concentrations were measured in duplicated samples by radioimmunoassay (RIA) methods, using commercial ^{125}I RIA kits.

Statistical analysis

All analyses were performed using GraphPad InStat® version 3.10 for Windows, 2009 (USA). Proportional data (mating acceptation, vulva appearance, percentage of high progesterone level) were analysed using the Fischer's Exact Test and the Chi-square test. Non-parametric tests for mean comparisons were used for plasma hormone level in each group (Wilcoxon matched-pairs signed ranks test), and between groups (Mann-Whitney Test). The values of plasma hormone levels were presented as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

The 48 h doe-litter separation improved the mating acceptance at day 11 PP (88.5 *vs.* 54.2%, P<0.005, Table 1). The sexual receptivity of DLS does depended on the time for which the female was presented to the male: 60 % (n=21) refused mating before opening the nest-box, while 80.9% of them (n=17) accepted the male after the nursing episode. The reason is likely related to the milk accumulation in the mammary gland during 48 hours which could provoke a feeling of discomfort (Rebollar *et al.,* 2004). There was no difference of mating acceptance at days 12-14 PP (75 *vs.* 43.7%, for DLS and C respectively, P>0.05). At day 11 PP, the percentage of the red or pink and turgid vulva was greater in DLS in comparison to C (54.3 *vs.* 20%, P<0.005). DLS does had the lowest percentage of white vulva (20 *vs*. 48.6%, P<0.05). The mating acceptance was maximal (high SR) when the vulva was red and turgid (100%, P<0.0001), or pink and turgid (93.3%, P=0.001) and minimal (low SR) when it was white (22.9 %, P<0.0001) (Figure 1). The percentage of does with medium SR (corresponded to the does which vulva was red or pink and no turgid, purple turgid and purple no turgid) was not different between C and DLS groups (P>0.05) (Figure 2). Diaz *et al.,* (1988) reported a positive influence on mating acceptance in lactating does with red and pink turgid vulvas, and high percentage of rejection for female with white vulva.

No difference (P>0.05) of E2, T, P4 and CT plasma levels was observed at day 9 PP between DLS and C groups. Plasma E2 levels in DLS group rose from day 9 to day 11 PP (200.4±24 *vs.* 289.3±43 pg/ml, P<0.01, respectively) contrary to C (216.4±31 *vs.* 230.9±30 pg/ml, P>0.05, respectively). Rebollar *et al.* (2006) described similar events in lactating multiparous does. The increased E2 concentrations likely reflect greater follicular steroidogenic activity (Ubilla *et al.,* 2000b). DLS does had also higher plasma testosterone level (103.9 ± 21 *vs.* 165.5 ± 27 pg/ml, P<0.005), compared to C ones (119.6±31 *vs.* 114.9±32 pg/ml, P>0.05). In our knowledge, the effect of temporary doe-litter separation on testosterone secretion has not been explored in the past. In the rabbit female, androgens induce sexual receptivity through their conversion to estrogens (Beyer and Mc Donald, 1973). The DLS does, after the separation, showed a significant increase (P<0.01) of plasma P4 levels from 0.65±0.08 at day 9 to 1.18±0.26 ng/ml at day 11 PP, compared to C does (0.74±0.12 *vs.* 0.99±0.21 ng/ml, P>0.05). These results are not in agreement with those reported by Ubilla *et al.*, (2001) who did not find any change in P4 plasma levels in multiparous does. Our results can, in part, be attributed to the does with "high P4 level" which is more frequent in primiparous than in multiparous, 32.5 % *vs.* 4%, respectively (Theau-Clément *et al.,* 2005). In our study, the percentage of high progesterone syndrome (P4>1ng/ml) was statistically not different between groups (30 *vs.* 40%, for DLS and C, respectively, P>0.05). The origin of high plasma P4 level in non-inseminated lactating does is likely due to spontaneous ovulation (Boiti *et al.,* 1996). An alternative explanation is that mild stress actives the hypothalamus-pituitary-adrenal axis to cause small progesterone secretion by the adrenal (Boiti and Canali, 1987), which could enhance gonadotropin release (Boiti, 2004). The DLS technique probably acts as mild stressor for the lactating doe, and induces progesterone and gonadotropin secretion. Blood cortisol levels did not vary in both groups between days 9 and 11 PP (101.3 ± 19 *vs.* 88.7±18 ng/ml for C, and 111.8±35 *vs.* 95.7 ± 41 ng/ml for DLS, P>0.05, respectively). In rabbits, cortisol and corticosterone represent a

Table 1: Mating acceptance rate and vulva characteristics in primiparous lactating does.

	CONTROL	**DLS**	*P value*
No. Primiparous does	*N = 35*	*N = 35*	
Mating acceptance (%):			
Day 11 PP	54.2[a]	88.5[b]	*P<0.005*
Days 12-14 PP	43.7[a]	75[a]	*NS*
Vulva characteristics at day 11 *postpartum* (%):			
Red or pink and turgid vulva	20[a]	54.3[b]	*P<0.005*
Red or pink, no turgid vulva	25.7[a]	17.1[a]	*NS*
Purple vulva	5.7[a]	8.6[a]	*NS*
White vulva	48.6[a]	20[b]	*P<0.05*
Turgid vulva	27.5[a]	54.3[b]	*P<0.05*

NS: not significant at P<0.05. Different letters in the same row indicate significant difference at P<0.05.

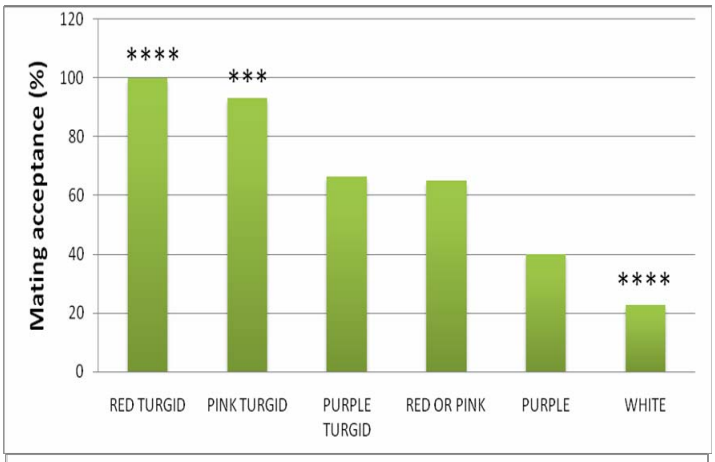

Figure 1: Influence of the colour and turgidity of the vulva on mating acceptation rate in primiparous does at days 11-14 PP (109 observations on 70 does of C and DLS groups. ***P<0.001; ****P<0.0001).

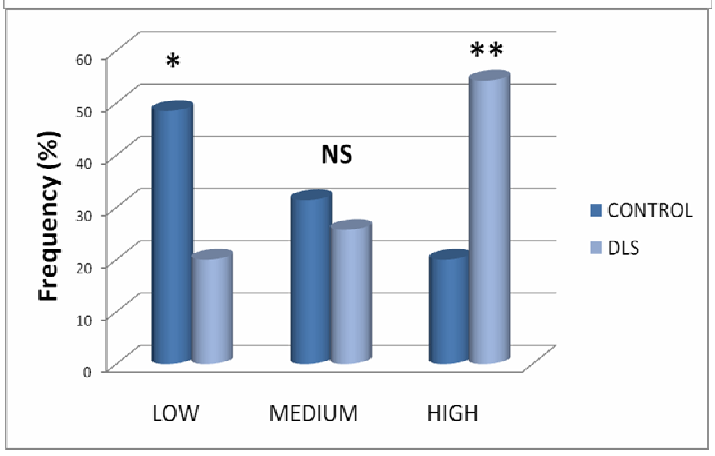

Figure 2: Sexual receptivity levels at day 11 PP in C and DLS groups (* P<0.05 ; ** P<0.005; NS: not significant at P<0.05).

good index of stress response (Szeto *et al.,* 2004). The stress induced by DLS was not effective in modifying cortisol secretion in biostimulated does. This can explained by the fact that the separation modality used in this work suppresses, for the does, visual perception of their litter, but permits acoustic and olfactory stimulations. No significant difference for cortisol levels was observed at day 11 PP between groups. The variation rates of the E2, T, P4 and CT plasma levels after the experimental treatment are reported in Figure 3.

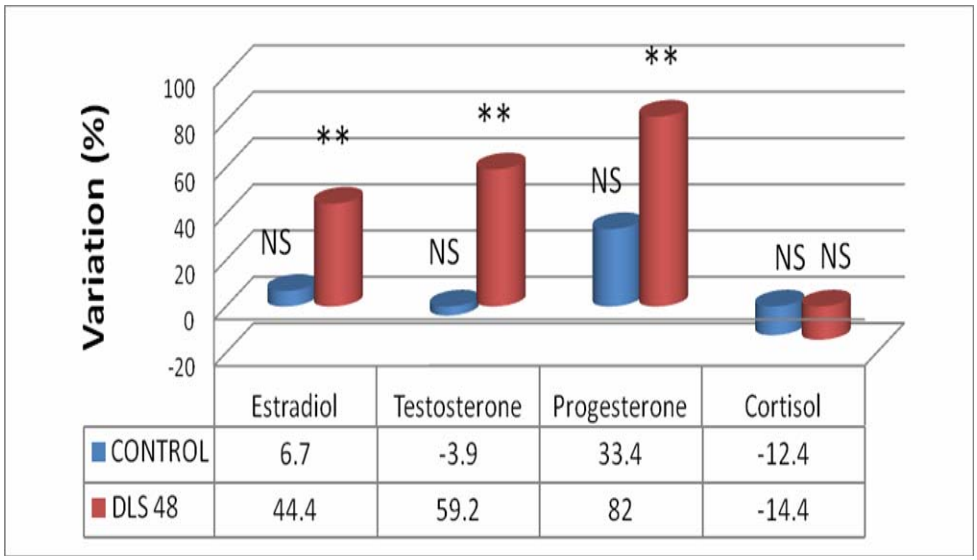

Figure 3 : Variation rates of plasma Estradiol, Testosterone, Progesterone and Cortisol concentrations after the experimental treatment (%). **(**NS: not significant , P>0.05; **, P<0.01).

CONCLUSION

The present results showed that the 48 hours doe-litter separation increase the mating acceptation of primiparous lactating does, submitted to a semi-intensive reproduction rhythm. After the experimental treatment, estradiol and testosterone plasma concentrations were significantly higher in DLS than in the control group. As estrogens, also androgen could play an important role in the oestrus induction after 48 hours doe-litter separation. In this study, the biostimulated does presented a significantly rise of plasma progesterone, but surprisingly, no modification of plasma cortisol levels. Further investigations are necessary to explore the effect of the DLS on the adrenal response of the doe.

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