

## HIGH PLASMATIC PROGESTERONE LEVELS AT INSEMINATION DEPRESS REPRODUCTIVE PERFORMANCE OF RABBIT DOES

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### ABSTRACT

The aim of this experiment was to quantify the frequency of the high progesterone syndrome at the moment of insemination and to study the influence of progesterone level (P4) on receptivity and reproductive performances of primiparous and secondiparous rabbit does. A total of 422 primiparous INRA 0067 were inseminated twice at an interval of 42 days. Prior to artificial insemination (AI), sexual receptivity of the does was tested in the presence of a vasectomised buck and, just after AI, blood samples were collected to determine progesterone concentration by RIA. The mean plasma progesterone concentration was  $1.8 \pm 3.4$  ng/ml and significantly depended on parity (primiparous:  $2.2 \pm 3.7$  ng/ml, secondiparous:  $0.9 \pm 2.2$  ng/ml,  $P < 0.001$ ). At the following AI, 78% of pseudopregnant rabbit does returned to the basal level of oestrous condition, demonstrating that the pseudopregnancy is a reversible process. The overall percentage of pseudopregnant does ( $P4 \geq 1$  ng/ml) was 25.9%, but it was differently ( $P < 0.001$ ) distributed between primiparous (31.2%) and secondiparous does (12.2%). Moreover, primiparous lactating females were more frequently pseudopregnant than non-lactating ones (36.5 vs. 18.9% respectively,  $P < 0.001$ ). The progesterone level of primiparous does was related to the lactation status ( $2.5 \pm 3.8$  vs.  $1.5 \pm 3.2$  ng/ml, respectively for lactating and non-lactating,  $P < 0.001$ ). The receptivity was highly related to the level of progesterone ( $P4 < 1$ : 74.1%,  $1 \leq P4 < 6$ : 79.1% vs.  $P4 > 6$ : 56.3%,  $P = 0.006$ ). Also the kindling rate was significantly influenced by progesterone concentrations. Non-pseudopregnant does ( $P < 1$  ng/ml) had the highest fertility rate (79.0%) but when the progesterone concentration increased from  $1 \leq P4 < 6$  to  $P4 > 6$  ng/ml, the fertility decreased from 68.1 to 37.4%, respectively ( $P < 0.001$ ). Consequently, the productivity at birth was highly depressed when the progesterone level was over 6 ng/ml (9.5 and 8.6 vs. 4.5 number of born alive rabbits/AI, for  $P4 < 1$ ,  $1 \leq P4 < 6$  and  $P4 > 6$  respectively,  $P < 0.001$ ). The productivity of pseudopregnant and non-receptive females was very poor in comparison with pseudopregnant receptive does (0.4 vs. 9.5 born alive rabbits/AI). The productivity at birth was also highly ( $P < 0.001$ ) influenced by the physiological status of the does. Primiparous non-lactating does produced the highest number of born alive rabbits/AI, whereas primiparous lactating does had the lowest productivity at birth (9.9 vs. 5.6), secondiparous lactating being intermediate (7.1). In conclusion, the high progesterone syndrome evaluated at the moment of insemination occurred in 25.9% of females and had a strong negative impact on receptivity and reproductive performance of rabbit does.

**Key words:** Rabbit, Pseudopregnancy, Progesterone, Physiological status, Receptivity.

### INTRODUCTION

In rabbit does, ovulation is a neuroendocrine reflex, typically triggered by mating or induced by exogenous GnRH administration. Therefore, functional *corpora lutea* (CL) should not be present in the ovaries of unmated rabbits or in the *post partum* period. On farms, Boiti *et al.* (1996) evidenced that up to 21% of multiparous rabbit does had abnormally high peripheral plasma progesterone concentration at the moment of artificial insemination (AI). Boiti *et al.* (2006) called this phenomenon the "high progesterone syndrome". On primiparous does inseminated from 0 to 19 days *post partum* or

2 days after weaning, Theau-Clément *et al.* (2000) showed that 20% of these females had 2 CL generations and high levels of progesterone (9.4 ng/ml). Only 22.9% of them were receptive at the time of AI, all ovulated but only one was fertile. In other studies, Theau-Clément *et al.* (2005) evidenced that the percentage of pseudopregnant does (plasma progesterone concentration >1ng/ml) varied according to the parity (nulliparous: 16.0%, primiparous: 32.5%, multiparous: 4.0%) and at a lower level than the lactation status (lactating: 18.1%, non-lactating: 10.4%,  $P < 0.005$ ). Consequently, the percentage of pseudopregnant does as well as the detrimental effect of progesterone on fertility seems to be related to the physiological status of rabbit does at the time of insemination.

The aim of this experiment was to quantify the frequency of “high progesterone syndrome” and to study the influence of progesterone level on receptivity and reproductive performance of primiparous and secondiparous rabbit does.

## MATERIALS AND METHODS

### Animals and experimental design

The experiment was performed at the INRA experimental Station (Auzeville, France). A total of 30 vasectomised bucks (receptivity tests) and 422 primiparous INRA 0067 (4 batches of 23 week old females) were used. The females were artificially inseminated (AI) two times at 42 days of interval. Prior to AI, sexual receptivity of the does was tested in the presence of a vasectomised buck as described by the International Rabbit Reproduction Group (2005). Inseminations were performed using a heterospermic pool diluted 7 times in Galap (IMV L’Aigle, France). Just after AI, blood samples were collected (1 ml) into heparinised vacutainers by venopuncture from the marginal ear vein. Then, ovulation was induced by i.m. injection of 0.8 µg of Buserelin acetate (0.2 ml Receptal®, Intervet, France). Litters were equalised to 9 rabbits at birth and weaning occurred at 28 days of lactation. Does were under a 16L:8D photoperiod and were fed *ad libitum* with a commercial diet containing 16.5% crude protein and 15.5% crude fibre. Water was provided *ad libitum*. Neither hormone nor biostimulation were used in this experiment to stimulate does receptivity.

After blood collection, plasma was separated by centrifugation and stored at -20°C until being assayed for progesterone concentrations by a specific and standardised RIA procedure (Boiti *et al.*, 1974). The sensitivity of the assay for a sample volume of 200 µl was 0.08 ng/ml. The evolution of body composition was controlled using TOBEC the day after each parturition or insemination and the day before weaning (results published by Bolet and Fortun-Lamothe, 2002 and Fortun-Lamothe, 2006).

### Statistical Analysis

The percentage of receptive does (lordosis position in the presence of a buck) and the fertility rate (kindling rate) were analysed as a Bernoulli variable (range 0-1) by analysis of variance like a classical continuous variable using the SAS statistics library. At the time of insemination, rabbit does were either primiparous, lactating or non-lactating, or secondiparous lactating. Therefore, litter size (total born, born alive and still born) and productivity at birth (number of born alive per AI) were analysed taking into account the fixed effect of the progesterone concentration (3 levels:  $P_4 < 1$ ,  $1 \leq P_4 < 6$  and  $P_4 > 6$  ng/ml), the physiological status of the does (3 levels: primiparous non-lactating, primiparous lactating, and secondiparous lactating) and their interaction. A previous analysis did not show a significant influence of the batch on receptivity, fertility, and litter size, and consequently this effect was not introduced in the model. The frequency of high progesterone syndrome was tested using a Chi square test.

**RESULTS AND DISCUSSION**

The results of 819 inseminations were analysed. At the first insemination, 146 does were pseudopregnant on the basis of plasma progesterone concentration (>1 ng/ml). At the second one, 42 days later, the progesterone level returned to the basal level (<1 ng/ml) for 78.0% of them. At the second insemination, 26 does non-pseudopregnant at the first A.I. became pseudopregnant.

*Pseudopregnancy frequency.* The percentage of pseudopregnant does was 25.9% and significantly linked to parity (primiparous: 31.2%, secondiparous: 12.2%, P<0.001). Moreover, primiparous females were more frequently pseudopregnant when lactating than non-lactating (36.5 vs. 18.9% respectively, P<0.001).

*Progesterone levels.* The mean plasma progesterone concentration was 1.8±3.4 ng/ml and significantly depended on parity (2.2±3.7 vs. 0.9±2.2 ng/ml, for primiparous and secondiparous respectively, P<0.001). On primiparous, the progesterone level was related to the lactation status (2.5±3.8 vs. 1.5±3.2 ng/ml, respectively for lactating and non-lactating, P<0.001).

*Influence of progesterone levels on reproductive performance of rabbit does* (Table 1). Whereas 67.8% of does were receptive at the moment of insemination, sexual behaviour was highly related to progesterone level (P4<1: 74.1%, 1≤P4<6: 79.1% vs. P4>6: 56.3%, P=0.006). In the same way, the kindling rate was significantly influenced by progesterone level. Non-pseudopregnant does had the highest fertility (79.0%) but when the progesterone concentration increased, the fertility decreased from 68.1 to 37.4% (for 1≤P4<6 and P4>6 ng/ml respectively, P<0.001). Consequently, due to the strong negative effect of progesterone on fertility, the productivity at birth was highly depressed when the progesterone level was greater than 6 ng/ml (9.5 and 8.6 vs. 4.5 number of born alive rabbits/AI, respectively for P4<1, 1≤P4<6 and P4>6, P<0.001). The interaction between progesterone level and the physiological status of the does was not significant for all of the measured parameters. However, the productivity was much higher in pseudopregnant receptive does than in pseudopregnant non-receptive does (9.5 vs. 0.4 born alive rabbits/AI). The difference between receptive and non-receptive does was weaker for non-pseudopregnant females (10.6 vs. 5.3 born alive rabbits/AI respectively for receptive and non-receptive).

**Table 1:** Influence of plasma progesterone levels and physiological status of does at insemination on reproductive performance. Results of variance analysis (least-squares means)

	Number	Receptivity (%)	Fertility (%)	Total born (n.)	Born alive (n.)	Still born (n.)	Productivity at birth <sup>(1)</sup>
General mean (%)	819	67.8	68.3	12.6	11.7	0.9	8.2
Residual standard error		44.3	42.9	3.4	3.9	2.2	5.8
R <sup>2</sup>		11.3	16.0	4.7	1.7	3.8	14.2
<b>Progesterone level (ng/ml)</b>		**	***	NS	NS	NS	***
P4 < 1	607	74.1 <sup>a</sup>	79.0 <sup>a</sup>	12.6	11.6	1.0	9.5 <sup>a</sup>
1 ≤ P4 < 6	105	79.1 <sup>a</sup>	68.1 <sup>b</sup>	13.0	12.4	0.6	8.6 <sup>a</sup>
P4 ≥ 6	107	56.3 <sup>b</sup>	37.4 <sup>c</sup>	13.2	11.6	1.6	4.5 <sup>b</sup>
<b>Physiological status</b>		***	***	T	T	*	***
Primiparous non-lactating	175	75.7 <sup>a</sup>	77.8 <sup>a</sup>	13.6 <sup>a</sup>	12.8 <sup>a</sup>	0.8 <sup>ab</sup>	9.9 <sup>a</sup>
Primiparous lactating	414	51.0 <sup>b</sup>	48.0 <sup>b</sup>	11.9 <sup>b</sup>	11.3 <sup>b</sup>	0.5 <sup>a</sup>	5.6 <sup>b</sup>
Secondiparous lactating	230	82.8 <sup>a</sup>	58.7 <sup>c</sup>	13.4 <sup>a</sup>	11.6 <sup>ab</sup>	1.7 <sup>b</sup>	7.1 <sup>c</sup>
<b>Progesterone*Physiological status</b>		NS	NS	NS	NS	NS	NS

NS: P>0.05, T: P<0.10, \*: P<0.05, \*\*: P<0.01, \*\*\*: P<0.001

Within columns, means with different letters are significantly different P<0.05

<sup>(1)</sup>Number of born alive rabbits/AI

*Influence of the physiological status of does on reproductive performances of rabbit does* (Table 1). The physiological status significantly affected all the performances examined. The primiparous lactating presented a lower receptivity rate (51.0 vs. 75.7 and 82.8% for primiparous non-lactating and secondiparous lactating, respectively, P<0.001) and consequently were less fertile than lactating does

(48.0 vs. 77.8 and 58.7% for primiparous and secondiparous respectively,  $P < 0.0001$ ). In the same way, the litter size of primiparous lactating does tended to be lower (total born: 11.9,  $P = 0.006$  and born alive: 11.3,  $P = 0.081$ ) compared to primiparous non-lactating and secondiparous lactating. In contrast, for primiparous lactating, the number of still born was lower (0.5,  $P = 0.035$ ) whereas an important mortality at birth was observed for secondiparous lactating (1.7 still born). Consequently, the productivity at birth highly ( $P < 0.001$ ) depended on the physiological status of does: primiparous non-lactating produced the higher number of born alive rabbits/AI whereas primiparous lactating had the lower productivity at birth (9.9 vs. 5.6), secondiparous lactating being intermediate (7.1). Receptivity highly influences the productivity at birth (10.6 vs. 3.6 born alive rabbits/AI). These results were in agreement with Theau-Clément *et al.* (2007).

Pseudogestation is a reversible process since at the following insemination, 78% of pseudopregnant rabbit does returned to the basal level.

In this study, the percentage of pseudopregnant does (25.9%) is in agreement with Theau-Clément *et al.* (2000, 2005) who obtained, on 11-day lactating primiparous does, respectively 46 and 32.5% of pseudopregnant does at the moment of insemination. In the same way, the progesterone level depends on parity (primiparous: 2.2 ng/ml, secondiparous: 0.9 ng/ml) as evidenced by Theau-Clément *et al.* (2005) who obtained a higher concentration of plasma progesterone in primiparous (2.4 ng/ml) than in nulliparous (0.4 ng/ml) or multiparous does (0.30 ng/ml). On pseudopregnant does, the plasma progesterone concentration reached  $6.4 \pm 4.0$  ng/ml whereas on 16 primiparous, Theau-Clément *et al.* (2005) obtained  $10.6 \pm 2.3$  ng/ml. A progesterone level greater than 6 ng/ml highly depresses the percentage of receptive does and fertility. The deleterious effect of progesterone on receptivity and fertility seems to vary with the experiment, since in the same conditions (pseudopregnant and primiparous 11-day lactating does), Theau-Clément *et al.* (2000, 2005) observed respectively only 25.0% of receptive does and no kindling, or 60% of receptive and 24.0% were fertile. The progesterone level did not significantly influence the litter size at birth, in agreement with the findings of previous authors.

Consequently, some ovulations of unknown origin occur more frequently on lactating does than on non-lactating ones. Several hypotheses have been proposed. Boiti *et al.* (1999) demonstrated that uterine infections increase the life span of CL which could explain high levels of progesterone at insemination. In our experiment this hypothesis is not probable since the females were young (only primiparous or secondiparous) and were conducted into four independent batches. Rommers *et al.* (2005) reported that pseudopregnancy occurs more frequently in the group-housing system (several does grouped in a same cage) than in the individual housing system (23.4 vs. 0%,  $P < 0.001$ ); this observation could explain the lower fertility in the former group (70.2 vs. 95.0%). Their results suggest that females may induce ovulation by mounting each other, but video tape recording evidenced no relationships between mounting behaviour and the high progesterone syndrome as originally suspected. In our experiment, all does were in a single cage. Moreover, no particular stress was registered in our experiment, and there was no relation between pseudopregnancy and buck proximity. However, the weight and the evolution of body composition were controlled using TOBEC the day after each parturition or insemination and the day before weaning. These measurements implied heavy manipulations (for each control, 3 passages in a measuring chamber) which could be a cause of spontaneous ovulation. Since these measurements were done systematically on all the does, whatever their physiological status, it could be suggested that the day after parturition, some does (particularly the lactating ones) were highly susceptible to spontaneous ovulation. According to this hypothesis, at A.I occurring 10 days later, the progesterone levels ought to be maximal (around 14 ng/ml) as demonstrated by Boiti *et al.* (2006). Since at the moment of insemination the average of progesterone concentration was 6.4 ng/ml, this hypothesis could not be ruled out.

## CONCLUSIONS

The present results confirm that more than 20% of rabbit does are pseudopregnant at the moment of insemination. The frequency of the high progesterone syndrome depends on the physiological status. High levels of progesterone, which are particularly frequent in primiparous lactating rabbit does (36.5%), have a deleterious effect on receptivity and fertility, leading to a 33% productivity decrease. The origin of the high progesterone syndrome still remains unknown. Further studies will be necessary to understand the physiological mechanisms underlying this phenomenon.

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## REFERENCES

- Boiti C., Ceccarelli P., Beghelli V., Daniotti P., Pennisi F. 1974. Messa a punto di un metodo per la determinazione radioimmunologica del progesterone plasmatico. *Anti Soc. It. Sci. Vet.*, 28, 366-371.
- Boiti C., Canali C., Monaci M., Stradaoli G., Verini Supplizi A., Vacca C., Castellini C., Facchin E. 1996. Effect of postpartum progesterone levels on receptivity, ovarian response, embryo quality and development in rabbits. *In: Proc. 6<sup>th</sup> World Rabbit Congress, Vol. 2, 45-50.*
- Boiti C., Canali C., Brecchia G., Zanon F., Facchin E. 1999. Effects of induced endometritis on the life-span of corpora lutea in pseudopregnant rabbits and incidence of spontaneous uterine infections related to fertility of breeding does. *Theriogenology* 52, 1123-1132.
- Bolet G., Fortun-Lamothe L. 2002. Relationship between body condition and reproductive performances in rabbit does. *In: Proc. 3rd meeting of workgroups 3 and 4. COST action 848, 2002 October, Ispra, Italy, Comm. n° 23.*
- Boiti C., Besenfelder U., Brecchia G., Theau-Clément M., Zerani M. 2006. Reproductive physiopathology of the rabbit does. *Recent Advances in Rabbit Sciences. Edited by L. Maertens and P. Coudert. 3-19.*
- Fortun-Lamothe L. 2006. Energy balance and reproductive performance in rabbit does. *Anim. Reprod. Sci.*, 93, 1-15.
- International Rabbit Reproduction Group 2005. Recommendations and guidelines for applied reproduction trials with rabbit does. *World Rabbit Science*, 13, 147-164.
- Rommers J., Boiti C., De Jong I., Brecchia G. 2005. Performance and behaviour of rabbit does in a group-housing system with natural mating or artificial insemination. *Reprod. Nutr. Dev.*, 46, 677-687.
- SAS 1993. Release 6.09 SAS *Technical Report P-243. SAS/STAT® Software. Cary, NC, USA.*
- Theau-Clément M. 2007. Preparation of the rabbit doe to insemination: a review. *World Rabbit Science*, 15, 61-80.
- Theau-Clément M., Boiti C., Mercier P., Falières J. 2000. Description of the ovarian status and fertilising ability of primiparous rabbit does at different lactation stages. *In: Proc. 7<sup>th</sup> World Rabbit Congress, Valencia, Spain, Vol. A, 259-266.*
- Theau-Clément M., Boiti C., Brecchia G. 2005. Characterisation of pseudopregnant rabbit does at the moment of artificial insemination. Preliminary results. *In: Proc. Joint Scientific meeting WG-1 (Reproduction) and WG-2 (Welfare). COST action 848, 2005 June, Palermo, Italy, Comm. n° 22.*

