

INFLUENCE OF DIFFERENT eCG DOSES ON SEXUAL RECEPTIVITY AND PRODUCTIVITY OF RABBIT DOES¹

Theau-Clément M.*, Lebas F.[†], Boiti C.[‡], G. Brecchia G.[‡], Mercier P.[§]

^{*}INRA. Station d'Amélioration Génétique des Animaux, BP 52627 - 31326 CASTANET TOLOSAN CEDEX, France.

[†]Cuniculture, 87a Chemin de Lasserre, 31450 CORRONSAC, France.

[‡]Dipartimento di Scienze Biopatologiche Veterinarie, Sezione di Fisiologia, Università di Perugia,
Via S. Costanzo 4, 06126 PERUGIA, Italy.

[§]INRA. Domaine Pluridisciplinaire du Magneraud, B.P. 52, 17700 SURGÈRES, France.

ABSTRACT: The aim of this study was to examine sexual receptivity and productivity of does injected 48 h before insemination with 8 or 25 IU of eCG (groups 8 and 25, respectively) in comparison with a control group (no injection, group 0). One hundred twenty four does were inseminated during 11 consecutive cycles (reproduction rhythm: 35 d; inseminations: 4 d *post partum* and weaning: 28 d *post partum*). In comparison with the control group, eCG significantly improves the receptivity of does (60.3 vs 80.5 and 79.8%; $P < 0.001$), kindling rate (70.4 vs 80.9 and 79.4%; $P = 0.030$) and the number of weaned rabbits/insemination (5.9 vs 7.1 and 7.1; $P = 0.008$) for group 0, 8 and 25 respectively, during the whole experiment. eCG efficiency is not obviously demonstrated for primiparous and non-lactating does. Authors conclude that only an 8 IU dose is able to improve 62% the productivity (measured as weaned rabbits per insemination) of multiparous and lactating does inseminated 4 d *post partum*.

Key words: rabbit, eCG, receptivity, fertility, prolificacy, productivity.

INTRODUCTION

In rabbit farms, because of an antagonism between lactation and reproduction, lactating non-receptive does at the moment of artificial insemination (AI) have poor performances. The sexual receptivity is an important criterion of prediction of the rabbit productivity, but it is not easy to identify it and in any case incompatible with cycled production (Theau-Clément, 2007). Consequently different strategies have been considered to improve rabbit sexual receptivity (Castellini, 1996; Theau-Clément, 2000, 2007). Thus, hormonal treatments have been largely used these last 15 years, particularly PMSG which is now called eCG (Equine Chorionic Gonadotrophin). This hormone is often used by breeders who have chosen to use AI and a single batch breeding system. If the positive effect of different eCG dose injections 48 h before insemination on rabbit does sexual receptivity is well known and durable (on 11 d lactating does; 10 IU: Bonanno *et al.*, 1991; 20 IU: Maertens, 1998; 25 IU: Theau-Clément and Lebas, 1996; 30 IU: Mirabito *et al.*, 1994), the repercussion on fertility becomes blurred after the 4th injection (40 IU: Canali *et al.*, 1991; 20 IU: Boiti *et al.*, 1995; 25 IU: Theau-Clément and Lebas, 1996, on 4 d lactating does; 25 IU: Rebollar *et al.*, 2006). Canali *et al.* (1989, 1991) and Boiti *et al.* (1995) established the relationship between the increase in anti-eCG antibodies to the wire of the injections and the loss of the effectiveness of the eCG. If the immune response depends on the number of injections and their interval, the relationship to the eCG dose has been hardly studied.

¹The results of that experiment were partially presented at the 7^{mes} Journées de la Recherche Cunicole (Lyon, 1998)

Correspondence: M. Theau-Clément, Michele.Theau-Clement@toulouse.inra.fr

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Moreover, the simultaneous administration of only 8 IU of eCG together with 4 IU with hCG, 48 h before insemination, every 35 d (4 d lactating does), makes it possible to improve the fertility of 9% (Davoust *et al.*, 1994). It is thus relevant to study the effectiveness of a small dose of eCG alone that could have the advantage of a reduction in the immunizing risk. In addition, Boiti *et al.* (1996) and Theau-Clément *et al.* (2000) observed at the moment of insemination some does having abnormal high plasma progesterone concentrations (>1ng/mL). These pseudopregnant does ovulate but have a poor sexual receptivity and are generally not fertilized. Because this abnormality was described as relatively frequent in primiparous does (from 20 to 31% according to Theau-Clément *et al.*, 2005), collection of blood samples was planned before the insemination in primiparous does in this study.

The aim of this study was to compare for the first time, the sexual receptivity, productivity and its components, of rabbit does receiving 8 or 25 IU of eCG, 48 h before insemination, in the particular case of a 35 d reproduction rhythm (i.e. AI at 4 d *post partum* and weaning at 28 d of age).

MATERIALS AND METHODS

The experiment was performed at INRA (Domaine Pluridisciplinaire du Magneraud, France). A total of 124 nulliparous INRA 0067 does (18 weeks old, issued from 2 batches with a 7 d interval) were inseminated every 35 d during 11 consecutive series, with Hyplus buck semen (Grimaud frères). At the first kindling, the does of the 2 batches were equally divided into 3 groups according to their lactation status (lactating or not), genealogy (sisters distributed in the different groups) and their body weight 3 weeks before the first insemination. A doe was definitively assigned to a group. Does were eliminated only for sanitary reasons and were systematically replaced by young does assigned to the group of the replaced doe.

Experimental design

Forty eight hours (± 2 h) before each insemination (except for the first one), the does of the eCG treated groups received a subcutaneous injection of 8 IU (group 8) or 25 IU (group 25) of eCG (Chronogest - INTERVET) in 1 mL of solvent. The does of the control group (group 0) did not receive any injection. Nulliparous does were never injected with eCG.

In order to study the serum progesterone level, two days before insemination, blood samples (3 mL in the marginal vein of the ear) were systematically taken from all the does (immediately before eCG injection for groups 8 and 25). The plasma was frozen (-20°C) and stored after centrifuging (20 min, 4000 rpm/min at 4°C). Plasma progesterone was measured by radioimmunoassay in accordance with the procedure described by Boiti *et al.* (1974). A doe was considered pseudopregnant if the serum progesterone level was higher than 1 ng/mL (Boiti *et al.*, 1999).

Prior to insemination, sexual receptivity of the does was tested in the presence of a buck. A doe was considered as receptive if she took a lordosis position. Inseminations were done 4 d *post partum* with heterospermic pools diluted 7 times in Dilap 2000. The ovulation was induced by the intramuscular injection of 0.2 mL of buserelin (Receptal®). No biostimulation was used in this experiment to induce does' receptivity. Free suckling was applied from birth up to weaning (28 d). The animals were housed in individual flat-deck cages under a 16L:8D constant lighting program and fed *ad libitum* with a commercial pellet diet containing 16.5% crude protein and 15.5% crude fibre. Water was provided *ad libitum*.

In order to analyse the ovarian response, fertilisation and the embryo survival, all the does were sacrificed 30 h (n=58) or 14 d (n=62) after the 11th insemination (Theau-Clément *et al.*, 2008). For that particular series, it has been considered that sacrificed does having at least one segmented egg 30 h after AI, or one

alive embryo 14 d after AI, were fertile, and their results were added to those of previous determinations of fertility (kindlings/AI).

Studied traits

The receptivity rate, fertility (number of kindling does/number of inseminated does), litter size at birth, at weaning (28 d), and the litter mean weight and individual mean weight at 28 d were studied. The global productivity was measured by the number of weaned rabbits/AI.

Statistical analysis

Because of a single batch breeding system (only 1 insemination every 35 d) and in order to have a perfect superposition of the number of inseminations and number of eCG injections, the data related to the does of renewal were excluded from the analysis. On none of the studied variables, a batch effect was evidenced (2 dates of birth of the females). Consequently, this effect was not included in further analysis. The analysis of reproductive performance at birth concerns 10 series and 9 series at weaning (Table 1).

The data were statistically analysed using the SAS statistics library (Statistical Analyse System, 1993). The effect of different eCG doses have been analysed by analysis of variance taking into account the fixed effect of treatment (3 levels: control, 8 IU, 25 IU), parity (2 levels: primiparous and multiparous), physiological status (2 levels: lactating and non-lactating) and their interactions. Taking into account the reproduction rhythm, at the moment of insemination, lactating does were 4-5 d lactating, while non-lactating does were separated from the previous litter for at least 10 d (does infertile after the previous AI). Receptivity rate and fertility were considered to be Bernoulli variables (range 0-1) and were analysed as traditional continuous variables. The ponderal productivity at weaning has been analysed introducing the litter size at weaning as a covariable.

RESULTS

Reproductive performances in relation with the number of eCG injections

Of the 124 lactating and primiparous does present at the first kindling (series 1), 70 were still present in the 11th series of AI. Doe survival was weaker for the control group (37.5%) than for treated groups (52.4% and 42.4% for groups 8 and 25, respectively) but differences were not significant.

Two days before insemination, an injection of 8 or 25 IU of eCG improves the sexual receptivity at the moment of insemination during the whole experiment period (Table 1 and Figure 1a). Nevertheless, it must be noted a less important effect of eCG on primiparous does (first injection). The fertility of does treated with eCG was higher than that of the does of the control group, the differences are generally maintained during the whole experiment. It must be noted that eCG has no effect on primiparous females (Figure 1b). During the experimental period, eCG does not regularly improve litter size at birth.

Overall efficiency of eCG treatments on receptivity induction and productivity

In comparison with the control group, 8 or 25 IU of eCG significantly ($P<0.001$) improves by 20 units of percentage the receptivity of does at the moment of insemination (Table 1). On the other hand, the increase of eCG dose has no significant effect on receptivity at the moment of insemination. The hormonal treatment and the parity interact: thus on primiparous, whatever the injected dose, eCG does not significantly improve rabbit does receptivity in comparison with the control group, whereas the effect is highly significant on multiparous (58.9 vs 90.3 and 86.1% respectively for groups 0, 8 and 25 IU; $P<0.001$, Figure 2). Moreover, a highly significant interaction between treatment and physiological status ($P<0.001$) evidences that, whatever the injected dose, eCG has no significant effect on receptivity induction of non-lactating does (Figure 3).

Table 1 : Reproductive performances traits of rabbit does in relation with eCG treatment, parity and the physiological status of the does at the moment of insemination.

Analysed series	No			9			9				
	No	Receptivity (%)	Kindling rate (%)	Born alive	Still born	Number observ.	Weaned/ kindling	28 d litter weight (g)	28 d kit. weight (g)	Number observ.	Weaned/ AI
General mean	967	73.2	76.9	9.5	0.7	660	8.7	4970	587	896	6.4
RSE		40.7	40.1	3.3	1.8		2.5	1099	72		4.2
R ²		0.16	0.10	0.04	0.03		0.05	0.02	0.50		0.12
eCG dose											
0 IU	357	60.3 ^a	70.4 ^a	9.4	0.8	207	8.6	4931	585	329	5.9 ^a
8 IU	299	80.5 ^b	80.9 ^b	9.8	0.5	220	8.9	5080	593	279	7.1 ^b
25 IU	311	79.8 ^b	79.4 ^b	10.0	0.7	233	9.1	5052	583	288	7.1 ^b
Parity											
Primiparous	187	68.6	68.5	9.8	0.4	119	8.8	5027	590	187	6.1
Multiparous	780	78.4	85.4	9.7	0.9	541	8.9	5015	584	709	7.3
Physiological status											
Lactating	751	67.0	65.1	9.0	0.5	489	8.3	4933	599	695	5.3
Non lactating	216	80.1	88.7	10.5	0.9	171	9.4	5109	575	201	8.1
Significance											
eCG dose		***	*	NS	NS	NS	NS	NS	NS	NS	**
Parity		**	***	NS	**	NS	NS	NS	NS	NS	**
Physiological status		***	***	***	*	***	***	NS	**	***	***
eCG dose×Parity		*	NS	NS	NS	NS	NS	NS	NS	NS	NS
eCG dose×Phys. stat.		***	NS	NS	NS	NS	NS	NS	NS	NS	NS
Parity×Phys. stat.		NS	*	NS	NS	NS	NS	NS	NS	NS	NS

RSE: Residual standard error.

Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and NS $P > 0.05$.Means in the same column with different superscripts differ significantly ($P < 0.05$).

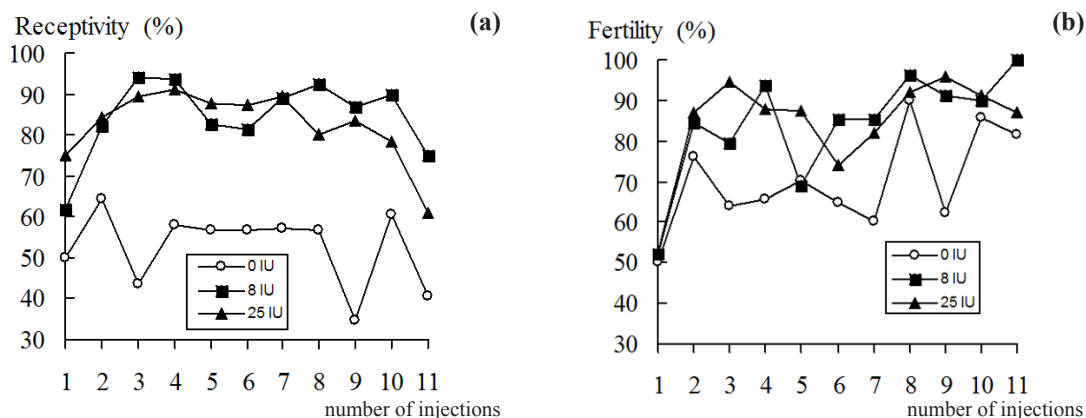


Figure 1: Influence of eCG dose (○, 0IU, ■, 8IU, ▲, 25IU) on the receptivity (a) and fertility (b) of does in relation with the number of eCG injections.

The injection of eCG significantly ($P=0.030$) improves the kindling rate, but the dose increase from 8 to 25 IU does not improve fertility (70.4 vs 80.9 and 79.4% respectively for groups 0, 8 and 25 IU). Primiparous and lactating does (primiparous or multiparous) are significantly less fertile than multiparous and non-lactating (respectively 68.5 vs 85.4%; $P=0.001$ and 65.1 vs 88.7%; $P<0.001$). The significant interaction between parity and physiological status highlights the difficulties of a doe simultaneously primiparous and lactating to be fertile (53.0%; $P=0.048$, Figure 4).

Whatever the amount injected, eCG does not have any significant effect on the litter size at birth or at weaning (Table 1). However, the distribution of the litter size was more homogeneous for group 8 IU (<8 born alive: 6.4, 3.3 and 7.0%; >12 born alive: 18.6, 9.1 and 19.7% for group 0, 8 and 25 IU, respectively; $P<0.05$). At birth and at weaning, lactating does have significantly lower litter sizes ($P=0.001$) than non-lactating does. Multiparous have more still born than primiparous ($P=0.009$). It is the same for non-lactating with respect to lactating ones ($P=0.043$).

The eCG treatments failed to have any ameliorative or deteriorating effect, neither on the litter weight at weaning, nor on the mean individual weaning weight of the kits. In addition, the young rabbits from lactating does are slightly heavier (+4.2%), than those resulting from non-lactating does ($P=0.002$).

Based on the first 9 series for which this criterion was calculable, the treatment by eCG makes possible to gain, whatever the amount used, 20% more weaned rabbits per AI (Table 1, $P=0.008$). Primiparous does produce significantly less weaned rabbits per AI than the multiparous (6.1 vs 7.3, respectively; $P=0.002$), and lactating does (primiparous and multiparous) also less than non-lactating ones (5.3 vs 8.1; respectively, $P=0.001$). Calculated on the arithmetic mean, the increase in the productivity consecutive to the injection of 8 IU of eCG is 6% for primiparous, 11% for non-lactating and 62% for the multiparous lactating does, while for 25 IU it is respectively; 6%, 16% and 67%.

Progesterone levels

On primiparous of this experiment, plasma progesterone levels have been studied on blood samples collected two days before insemination. From the 124 does, 22 (17.7%) were pseudopregnant (19.0, 21.4, and 12.5%, for groups 0, 8 and 25 IU, respectively). A new analysis of variance has been done on primiparous does after exclusion of pseudopregnant ones. Whatever the amount injected, eCG failed to improve any of the productivity components in non-pseudopregnant rabbit does. As a consequence, the

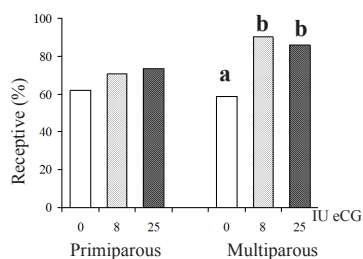


Figure 2: Influence of eCG dose on the percentage of receptive does in relation with their parity. ^{a,b} Bars no sharing a common superscript differ significantly ($P < 0.05$).

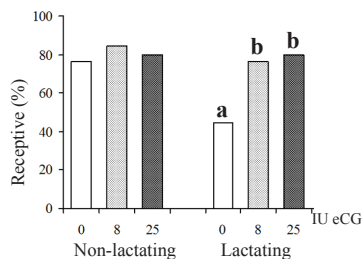


Figure 3: Influence of eCG dose on the percentage of receptive does in relation with their physiological status. ^{a,b} Bars no sharing a common superscript differ significantly ($P < 0.05$).

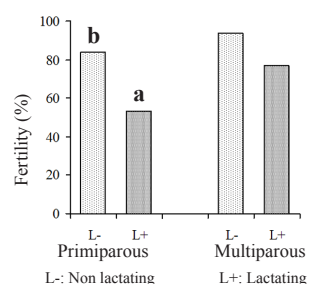


Figure 4: Influence of parity and physiological status on fertility. ^{a,b} Bars no sharing a common superscript differ significantly ($P < 0.05$).

relatively high proportion of pseudopregnant primiparous does is not the explanation of the absence of eCG effect in this group of rabbit does.

When receptivity is introduced as a fixed effect in the model, all the direct eCG effects on reproductive performance are suppressed. This observation confirms that this molecule mainly acts by inducing the rabbit does sexual receptivity.

DISCUSSION

In opposition with the results of Rebollar *et al.* (2006), an eCG injection does not significantly influence the rate of survival of the does in these experimental conditions. As different authors have already evidenced (Maertens *et al.*, 1983; Bonanno *et al.*, 1991, 1996; Mirabito *et al.*, 1994; Theau-Clément and Lebas, 1996; Alabiso *et al.*, 1998), the eCG injection 48 h before insemination durably improves rabbit does sexual receptivity. In agreement with Mirabito *et al.* (1994) and Alabiso *et al.* (1998), eCG does not improve receptivity on non-lactating does. Indeed, these does have optimal reproductive potentialities (receptivity > 80%). In this study, eCG does not influence primiparous receptivity.

Whatever the number of eCG injections, the fertility is improved. The decrease of eCG efficiency after the 4th injection demonstrated by Canali *et al.* (1991) and Theau-Clément and Lebas (1996) is not obtained in these experimental conditions. However, it must be underlined the weak effect of eCG to improve the fertility of primiparous or non-lactating does in opposition with Bourdillon *et al.* (1992), Davoust *et al.* (1994) and Maertens (1998). The hypothesis was done that pseudopregnant does could be responsible of the eCG inefficacy to induce receptivity and consequently fertility. The elimination of does detected as pseudopregnant (blood plasma progesterone concentration 48 h before AI > 1 ng/mL) allows to confirm the lack of eCG efficiency on the fertility of primiparous does in our study.

As Castellini *et al.* (1991), Maertens and Luzi (1995), Mirabito *et al.* (1994), but contrary to Bourdillon *et al.* (1992), Davoust *et al.* (1994), Theau-Clément and Lebas (1996), Maertens (1998) and Rebollar *et al.* (2006), eCG does not improve the litter size at birth or at weaning, nor the kits weight at weaning. In the conditions of this experiment, the numerical and ponderal productivity is not influenced by eCG treatment.

Whatever the component of the productivity taken in consideration, a moderate amount of eCG (8 IU) gives reproductive performances at least as satisfactory as a higher amount (25 IU). This clearly confirms

the results obtained by Davoust *et al.* (1994) with an association of 8 IU eCG + 4 IU hCG injected to rabbit does conducted with the same reproduction rhythm (AI every 35 d). Moreover, the does treated with only 8 IU of eCG have litter sizes more homogeneous than those treated with 25 IU of PMSG or not receiving injection. This observation is in opposition with Maertens and Luzi (1995) and Rebollar *et al.* (2006) using 30 or 25 IU, and a 42 d or 35 d reproduction rhythm, respectively.

However, the improvements observed with the lowest eCG dose may be related with the specific status of does treated only 4 d on average after parturition. Effectively, Theau-Clément and Mercier (2003) observed that 8 IU of eCG is not a sufficient amount to improve the fertility of 11 d lactating does.

This study confirms the conclusions of Theau-Clément and Lebas (1996): eCG acts by increasing the proportion of receptive does. This experiment confirms too the difficulties of primiparous (Chmitelin *et al.* 1990) and lactating-non-receptive does (Theau-Clément and Roustan, 1992; Alabiso *et al.*, 1996) to reach high levels of productivity.

CONCLUSION

When applying a 35 d reproduction rhythm (AI at 4 d *post partum* and weaning at 28 d *post partum*), eCG does not affect the survival rate of does nor the litter size or young growth but increases receptivity and by this way rabbit does' fertility. Only 8 IU eCG are sufficient to improve by 62% the productivity of 4 d lactating multiparous does. This improvement is obtained quite exclusively by increasing the proportion of receptive does in this group. The improvement is definitely weak for primiparous or non-lactating does probably blaming the economic interest of the use of eCG for these two groups of females.

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