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INFLUENCE OF DIFFERENT eCG DOSES ON THE RABBIT DOE OVARY RESPONSE, FERTILISING APTITUDE AND EMBRYO DEVELOPMENT

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Abstract: The aim of this experiment was to compare the ovary response, the fertilising aptitude and the embryo development of multiparous rabbit does having received during the whole career 0 (control: no injection, group 0), 8 or 25 IU of eCG (groups 8 and 25, respectively) 48 h before each 4 d *post partum* insemination. After the 11th series of insemination, two groups of 60 does were sacrificed 30 h or 14 d after insemination. The percentage of ovulating females does not vary according to the eCG treatment, but the ovulation rate (number of corpora lutea per ovulating does) and the fertilising rate (number of segmented ova/number of corpora lutea×100) increases with eCG dose [10.2, 11.1, 12.3 corpora lutea (*P*=0.011); 61.6 *vs* 97.5, 81.2% (*P*<0.001), for groups 0, 8 and 25 IU respectively]. In a same way, at 14 d of pregnancy, the embryo survival (1-(number of corpora lutea – number of alive embryos/ number of corpora lutea)) increases with the injected amount of eCG (55.1 *vs* 69.9 and 83.1% for groups 0, 8 and 25, respectively, *P*=0.023). Moreover, eCG completely removes the fertilising failure independent of ovulation. Compared with the other does, lactating-non-receptive does have a lower fertilisation rate (70.0 *vs* 90.2%, *P*=0.008) and a weaker embryo survival (55.3 *vs* 83.5%, *P*<0.001). It is concluded that with intensive reproduction rhythm (4 d *post partum* insemination), an 8 IU of eCG injected 48 h before insemination is enough to enhance the ovulation rate, the embryo survival and the fertilisation rate of multiparous does.

Key words: rabbit, eCG, ovary response, fertilising aptitude, embryo development.

INTRODUCTION

Boiti *et al*. (1995) and Stradaioli *et al*. (1994, 1997) evidenced that an at least 3 times repeated 20 IU eCG injections 72 h before insemination (11 or 26 d post partum, respectively) increase the number of haemorrhagic follicles on rabbit ovaries and decreases the in vitro development of fertilised ova. In a context of frequent use of eCG in rabbit farms, Theau-Clément *et al*. (2008) have compared the sexual receptivity, productivity and its components of rabbit does receiving 0, 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). The aim of this study was to compare in these conditions, the ovary response, the fertilising aptitude and the embryo development of multiparous does having received during their whole reproductive career, 8 or 25 IU of eCG, in comparison with a control group (no injection).

MATERIALS AND METHODS

Experimental design

The experimental design has been described in more details in our previous article (Theau-Clément *et al*., 2008). A total of 124 primiparous INRA 0067 does were 4 d post partum inseminated every 35 d with

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Hyplus buck semen (Grimaud frères) during 11 consecutive series of AI. Prior to insemination, sexual receptivity of the does was tested in the presence of a buck. Culled does, exclusively for pathological reasons, were replaced by young does. The does 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. Forty eight hours before each insemination, 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 control group did not received any eCG injection (group 0). Inseminations were done 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 nursing was applied. The animals were housed in individual flat-deck under a 16L:8D constant lighting program and fed *ad libitum* with a commercial pellet diet containing under 16.5% crude protein and 15.5% crude fibre. Water was provided *ad libitum.*

The day of the 11th insemination of the initial does, after the receptivity test, 120 multiparous does were distributed in two identical sub-groups within each eCG goup (n=20) according to their physiological status (lactation and receptivity) and the number of kits per litter (born from the 10th insemination). The replacement does have produced a minimum of 3 litters, i.e. they received a minimum of 3 eCG injections in their life in the 2 eCG groups. The first sub-groups of does were sacrificed 30 h after insemination, the second ones, 14 d after insemination.

Studied traits

The experimental herd was composed by 70 does remaining from the initial herd and 50 renewal does. The influence of eCG treatments and the physiological status of the does at the moment of insemination have been studied on their ovulation potentialities (120 does), on the ovarian response and fertilising aptitude observed 30 h after insemination (58 does), and on the embryo development at 14th d of pregnancy (62 does).

For the 58 does sacrificed 30 h after insemination, the ovaries were removed to measure the percentage of ovulating does, the number of corpora lutea, of preovulatory follicles and haemorrhagic follicles (if diameter greater than 1 mm, Mariana *et al*., 1989). Both of the oviducts and uterine horns were repeatedly flushed with 5 mL of saline solution until no oocyte (no segmented egg) or segmented ova were found. All collected oocytes and segmented ova were counted and observed under a microscope (×50). These observations have allowed analysing the ovulation rate (number of corpora lutea per ovulating does×100) and the fertilising rate (estimated on ovulating does by the number of segmented eggs×100/number of corpora lutea). For the 3 sub-groups sacrificed 14 d after insemination, the number of corpora lutea was measured after removal of the 2 ovaries, and in addition the number of alive and dead embryos and the visible sites of implantation deprived of foetus were counted. The embryo survival (1-(number of corpora lutea – number of alive embryos/number of corpora lutea)) was estimated.

Statistical analysis

Data were statistically analysed using the SAS statistics library (Statistical Analyse System, 1993). The effect of different eCG doses on the ovarian response, the fertilisation aptitude and embryo viability of multiparous does have been analysed by analysis of variance, taking into account the fixed effect of the treatment (3 levels: control, 8 IU, 25 IU) and the physiological status of the does at time of insemination (2 levels: lactating-non-receptive, others) and the interaction treatment×physiological status. Indeed, Theau-Clément (2007) evidenced significantly weak performances of 4 d lactating non-receptive does, due to a lactation-reproduction antagonism. Ovulation and fertilisation rates were considered to be Bernoulli variables (range 0-1) and analysed as traditional continuous variables.

RESULTS

Ovulation

The percentage of ovulating females does not significantly vary according to the eCG treatment (Table 1). But the ovulation rate increases with eCG dose (10.2, 11.1, and 12.3 corpora lutea, respectively for groups 0, 8 and 25 IU respectively; P=0.011).

The ovulation frequency of lactating-non-receptive does is significantly weaker than for the others physiological status (88.4 *vs* 97.8% respectively; P=0.035) while the ovulation rate does not vary according to the physiological status of the does (10.7 and 11.5) at the moment of insemination.

Ovary response and fertilisation studied 30 h after 4 d post insemination.

Whereas the number of preovulatory follicles decreases according to the eCG amount injected, in parallel, the number of corpora lutea increases, however, the differences are not significant (Table 2). The number of haemorrhagic follicles does not significantly vary according to the treatment.

Does of the control group produce significantly less collected eggs (7.7 *vs* 10.9 and 10.0 for groups 0, 8 and 25 respectively; P=0.026) and segmented ones (6.6 *vs* 10.9 and 9.7 for groups 0, 8 and 25 respectively; P=0.004). The fertilisation rate is significantly improved by eCG treatments (P=0.001). Although the numerical variation is not significant, compared to group 25, the does treated with 8 IU of eCG record the best performance (97.5 and 81.2 *vs* 61.6%, for groups 8, 25 IU and the control). It is however necessary to underline a significant interaction between the group and the physiological state of does on the fertilisation rate. Indeed, the fertilisation rate is significantly weaker on lactating non-receptive does of the control group compared to the others (37.4 *vs* 85.8%; P=0.014, Figure 1), even among the fertile does (having at least one segmented egg) only 56.1% of eggs are fertilized (data not shown).

Table 1: Influence of eCG does and physiological status of does on the ovulation frequency and ovulation rate.

	Number	Ovulation frequency (%)	Corpora lutea
General mean	120	95.0	11.4
RSE		21.7	2.6
R^2		0.05	0.15
eCG dose			
0 IU	39	97.6	10.2[a]
8 IU	39	93.3	11.1[ab]
25 IU	42	88.4	12.3[b]
Physiological status			
L+ R−	41	88.4	10.7
Others	79	97.8	11.5
Significance			
eCG dose		NS	*
Physiological status		*	NS

RSE: Residual standard error, L+R−: lactating non receptive.
Significance: *P<0.05, and NS P>0.05
eCG×Physiological status interaction was not significant for both traits.
Means in the same column with different superscripts differ significantly (P<0.05).

Table 2: Influence of eCG dose and physiological status of does on the ovarian response and fertilisation 30 h after insemination.

		Ovarian response			Fertilisation		
	Number	Preovulatory follicles Ø>1mm	Haemorrhagic follicles Ø>1mm	Corpora lutea	Collected eggs	Segmented eggs	Fertilisation rate (%)
General mean	58	6.7	0.5	11.2	9.9	9.4	81.6
RSE		4.3	1.0	2.9	3.5	3.7	0.3
R^2		0.11	0.11	0.16	0.30	0.36	0.41
eCG dose							
0 IU	19	8.4	1.0	9.7	7.7[a]	6.6[a]	61.6[a]
8 IU	19	6.1	0.3	11.2	10.9[b]	10.9[b]	97.5[b]
25 IU	20	5.4	0.3	12.0	10.0[b]	9.7[b]	81.2[b]
Physiological status							
L+ R-	19	6.9	0.6	10.4	8.0	7.5	70.0
Others	39	6.4	0.5	11.5	11.0	10.7	90.2
Significance							
eCG dose		NS	NS	NS	*	**	***
Physiological status		NS	NS	NS	**	**	**
eCG dose×Physiological status		NS	NS	NS	NS	NS	*

RSE: Residual standard error, L+R−: lactating non receptive.
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).

Lactating-non-receptive does produce a similar number of preovulatory follicles, haemorrhagic follicles and corpora lutea compared with does at others physiological status (Table 2), but they have significantly less segmented ova (7.5 *vs* 10.7). Consequently, the fertilisation rate is significantly depressed (70.0 *vs* 90.2% respectively; P=0.008).

Embryo development measured 14 d after 4 d post insemination.
The number of alive embryos and total implantation sites increases significantly (P<0.05) according to the eCG amount (Table 3) but the number of dead embryos or implanted and then degenerated does not significantly vary according to the injected eCG dose. Consequently, the embryonic survival increases with the injected amount of eCG (55.1, 69.9 and 83.1% for groups 0, 8 and 25, respectively; P=0.023).

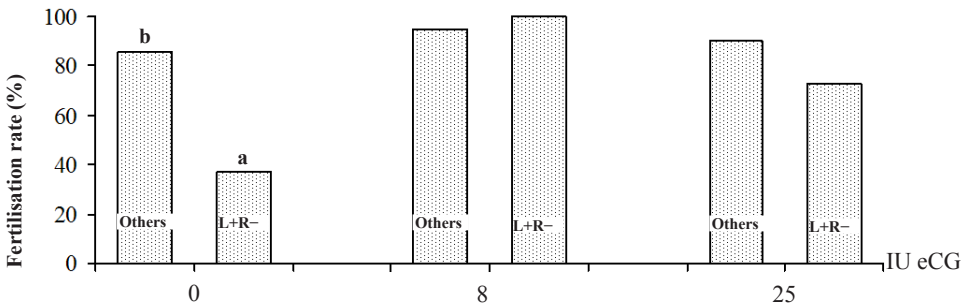

Figure 1: Fertilisation rate in relation with eCG dose and the physiological status of does. L+R−: Lactating non-receptive doe. [a,b] Bars no sharing a common superscript differ significantly (P<0.001).

Table 3: Influence of eCG dose and physiological status of does on the ovarian response and embryo development 14 d after insemination.

	Number	Corpora lutea	Alive embryos	Dead embryos	Implantation sites without embryo	Total implantation sites	Embryo survival (%)
General mean	62	11.6	8.8	0.2	0.3	9.3	74.1
RSE		2.4	3.5	0.4	0.7	3.4	25.4
R^2		0.19	0.33	0.15	0.06	0.37	0.42
eCG dose							
0 IU	20	10.8	6.3[a]	0.2	0.2	6.7[a]	55.1[a]
8 IU	20	11.1	7.8[a]	0.2	0.3	8.3[ab]	69.9[ab]
25 IU	22	12.6	10.5 [b]	0.3	0.3	11.0 [b]	83.1 [b]
Physiological status							
L+R−	22	11.1	6.3	0.1	0.1	4.7	55.3
Others	40	12.0	10.1	0.3	0.4	10.3	83.5
Significance							
eCG dose		NS	*	NS	NS	**	*
Physiological status		NS	***	NS	NS	***	**
eCG dose×Physiological status		NS	NS	NS	NS	NS	NS

RSE: Residual standard error, L+R−: lactating non receptive.
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).

Lactating-non-receptive does have less alive embryos (6.3 *vs* 10.1 respectively; P<0.001), implanted embryos (4.7 *vs* 10.3 respectively; P=0.001), result of a weaker embryo survival (55.3 *vs* 83.5% respectively; P<0.001).

It was not possible to analyse the fertilising failure independent of the ovulation. Indeed, only 5 does lactating-non-receptive and 2 does non-lactating-non-receptive of the control group did not have any segmented or alive embryo (30 h or 14 d after insemination, respectively). Only the non-receptive does at the time of insemination thus present this phenomenon. Whatever the injected does, eCG thus completely removed the fertilising failure independent of ovulation.

DISCUSSION

Little bibliography exists related to eCG effects on the ovarian response, fertilising aptitude and the embryo development, moreover, the conclusions are sometimes divergent. Among the sources of variation of the different experimental conditions, it is necessary to mention the genetic type of the does, the breeding system and the conditions of injection (interval between injections, interval between injection and insemination, eCG dose, administration way, injected volume…). In the objective to exacerbate the immunizing risks, a 35 d reproduction rhythm was chosen in the present study, whereas a 42 d rhythm is applied in most of the quoted studies. In the present test, the eCG injection was practised 48 h before insemination, the interval was often 72 h in the other cases, interval generally not followed by an increase of reproductive performances. In the present study, eCG was injected in 1mL of solvent by subcutaneous way, but in quoted work, the injections are intramuscular and generally volume remains unknown.

Considering the whole of the experimental does, eCG does not influence the percentage of ovulating does. On the contrary, the ovulation rate increases with the eCG dose by 8.8 and 20.6% for 8 and 25 IU, respectively. Similarly, injecting repeatedly (at least 3 times) 20 IU of eCG 72 h before insemination on 11 d post partum multiparous does, Boiti *et al*. (1995) improved but to a higher extend (61%) the number of corpora lutea (14.2 *vs* 8.8 for the control group without injection).

Bonanno *et al*. (1990) evidenced that a 20 IU eCG injection before a GnRH injection increases the follicular growth. In the conditions of the present experiment, 30 h after insemination, the follicular potential (follicles with a diameter greater than 1mm) represented by the sum of the whole of the follicles having burst (measured by the number of corpora lutea), plus the preovulatory follicles and the haemorrhagic follicles, varies little according to the eCG dose (19.1, 17.6 and 17.7 for 0, 8 and 25 IU, respectively). Consequently, in agreement with these last authors, eCG does not act directly on follicular recruitment, but increases the follicles growth increasing by this way oestrogens secretion and consequently the sexual receptivity of the does. In agreement with Bonanno *et al*. (1990) and Stradaioli *et al*. (1993) but contrary to Boiti *et al*. (1995) and Stradaioli *et al*. (1994, 1997), the increase in the number of hemorrhagic follicles after eCG treatment is not confirmed (only 0.3 hemorrhagic follicle on average for the treated groups).

In agreement with Boiti *et al*. (1995), the number of segmented eggs is improved by the treatment. At the opposite, the negative effect of eCG on the early embryonic development (30 h after AI) is not confirmed, on the contrary at mid-pregnancy (14 d after AI), the embryonic survival is improved by 8 or 25 UI eCG. Since the number of dead embryos and the number of implantation sites without embryo does not vary according to the treatment, the positive eCG effect on the embryo survival could be the consequence of the fertilisation rate improvement. The fact that the litter size at birth is not significantly modified by the treatment (9.4, 9.8 and 10.0 for 0, 8 and 25 IU eCG, respectively, Theau-Clément *et al.*, 2008), suggests that the foetal mortality (between 14 d and kindling) could be higher for treated does. Gathering experimental does sacrificed 30 h or 14 d after insemination, the productivity by insemination measured by the number of segmented eggs (or alive embryos at 14 d of pregnancy), is improved by eCG treatment (6.4 *vs* 8.9 and 9.0 for 0, 8 and 25 IU, respectively) but without difference between the 2 doses. Consequently, if applying a 35 d reproduction rhythm, the use of an eCG dose higher than 8 IU is not justified.

This work confirms the observations of Theau-Clément and Roustan (1992), Castellini and Lattaioli (1999) and Theau-Clément (2007) who highlighted the difficulty of a rabbit doe being simultaneously lactating and non-receptive at insemination time (particularly for 4 d lactating doe) to be as fertile as the others. This study clearly evidences that 4 d lactating and non-receptive females not only ovulate less frequently, but have a lower fertilisation rate and consequentlely a lower number of alive embryos at mid-pregnancy leading to an important decrease of global productivity (6.0 *vs* 10.2 segmented ova or alive embryos at mid-pregnancy).

CONCLUSION

This experiment confirms the difficulties of 4 d lactating and non-receptive does, to reach high levels of productivity. At the ovary level, on multiparous rabbit does, with intensive reproduction rhythm (35 d), an eCG injection improves the growth and probably the final maturation of the follicles, explaining the improvement of the ovulation rate, fertilisation rate, embryo survival at mid-pregnancy and the total lack of pregnancy failures independent of ovulation. Although 25 IU eCG was able to significantly improve the ovulation rate, the number of alive embryos and consequently the embryo survival at mid-pregnancy, the global productivity and the reproductive performances measured at birth were similarly improved with 8 or 25 IU eCG (Theau-Clément *et al*., 2008). Consequently, it is concluded that with intensive reproduction rhythm (4 d post partum insemination), an 8 IU of eCG injected 48 h before insemination is enough to enhance the rabbit reproductive performance.

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