

# OVULATION INDUCTION IN RABBIT DOES BY INTRAVAGINAL ADMINISTRATION OF THE GnRH ANALOGUE [des-Gly10, D-Ala6]–LHRH ETHYLAMIDE: FIELD TRIAL

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## ABSTRACT

In the rabbit doe, ovulation does not occur spontaneously, but it has to be induced through a neurohormonal reflex, which is initiated during mating. So, when using AI, in the absence of a male, ovulation has to be induced by artificial methods. The ovulation inducing method most frequently used is an intramuscular injection of a synthetic analogue of GnRH or gonadorelin. An alternative way, demonstrated in previously studies, is the intravaginally administration of the hormone.

This study aimed to evaluate the efficacy of GnRH analogue [des-Gly10, D-Ala6]–LHRH ethylamide, administered intravaginally, to induce ovulation in rabbit does submitted to AI. In this experiment, a large scale field trial was done to test the use of 25 µg of GnRH analogue [des-Gly10, D-Ala6]–LHRH ethylamide vehiculated in the seminal dose (n=270) against 20 µg of gonadorelin via intramuscular (n=270). Fertility was higher (P<0.05) when ovulation was induced by intravaginal administration of [des-Gly10, D-Ala6]–LHRH ethylamide (91.1% vs 85.6%). Prolificity or mortinatality were never affected by the ovulation induction treatments.

It was concluded that GnRH analogue [des-Gly10, D-Ala6]–LHRH ethylamide can be used for ovulation induction in rabbit does vehiculated in the seminal dose, with better AI results as those obtained with gonadorelin administered intramuscularly.

**Key words:** Rabbit, [des-Gly10, D-Ala6]–LHRH ethylamide, Ovulation induction, Intravaginal administration.

## INTRODUCTION

The use of artificial insemination (AI) in rabbit farms has become a common practice in European countries, being currently used in more than 80% of the Spanish commercial rabbit farms. Rabbits are induced ovulators and, therefore, when using AI, ovulation has to be induced by artificial methods. The ovulation inducing method most frequently used is an intramuscular injection of GnRH or its syntethic analogues (Michelmann and Paufler, 1973; Theau-Clément *et al.*, 1990).

In most rabbit farms, GnRH injection is usually done by the farmer himself, with a certain risk of misuse, and increasing the time needed for the AI of each doe. In a previous study, we have shown that the GnRH agonist buserelin can be used by intravaginal administration, adding 16 µg of the hormone to the seminal dose, with similar results as those obtained by intramuscular injection of 0.8 µg buserelin (Quintela *et al.*, 2004). With this method, potential mistakes derived from the hormone not being correctly injected into the muscle are eliminated, since the hormone can be added in the AI Center when preparing the seminal doses and it reduces the time spent by the farmer on the AI of each doe.

In the present study, that is a continuation of the previous one, a different GnRH agonist was studied as potential method for ovulation induction in rabbits by intravaginal administration: the GnRH

agonist [des-Gly10, D-Ala6]–LHRH ethylamide, that has lower potency than Buserelin (0.7 times) and is about 14 times more potent than Gonadorelin (Conn and Crowley, 1991). The GnRH agonist was first tested, at different concentrations, in a small number of experimental rabbit does (Quintela *et al.*, 2007), and then, a large scale field trial was done in a commercial farm using the concentration that, in the preliminary experiments, produced the best results.

## MATERIALS AND METHODS

### Animals and experimental design

Five hundred and forty multiparous does (Hyplus strain PS19, Grimaud Frères, France) located in the original industrial farm (COGAL, SL), were randomly chosen for a field trial. The 540 does were randomly divided into 2 groups of 270 does each. Environmental and feeding conditions as well as the reproductive management of the does were as previously described (Quintela *et al.*, 2004). Briefly, in the farm the does were submitted to 42-day AI cycles. For each cycle they were treated hormonally to synchronize the oestrus. The hormonal treatment consisted of 20 IU PMSG (Folligon, Intervet, Salamanca, Spain), injected intramuscularly in a volume of 0.4 ml, 48 h before AI. All the does were exposed to a light intensity of 70 lux, with an artificial lighting program of 12 h L (light)/12 h D (dark), which was changed to 16 h L/8 h D six days before AI. During the 4 days following AI, light was reduced at 1 h per day, to recover the 12h L/12 h D photoperiod. Controlled suckling was applied to all does from 0 to 10 days post partum, by keeping the nest door closed and only opening it every 24 h, at 12:00 h for 5-10 min, to allow the kits to suck once a day. Controlled suckling before AI is thought to increase does sexual receptivity at the next AI, as it decreases the antagonism between prolactin and gonadotrophin release. The day of AI (day 11 post partum) suckling was delayed until 17:00 h, 5–10 min before performing the AI. This made a 30-hmother-litter separation.

The 2 groups of 270 multiparous does each were inseminated on 6 different days between october and november 2005 (45 does/day per group). Only 1 AI was done on each doe (n=270) in the context of this experiment. To induce ovulation, the does of the control group received 20 µg/doe of gonadorelin (InduceL GnRH: 20 µg·ml<sup>-1</sup>), 1 ml administered intramuscularly and, the does of the experimental group received 25 µg of [des-Gly10, D-Ala6]–LHRH ethylamide (L-4513, Sigma, St. Louis, MO, USA). The powder was diluted in saline solution (1 mg·ml<sup>-1</sup>) just before AI and was administered intravaginally by adding 25 µL of GnRH solution per insemination dose. The time lapse between GnRH addition to the semen and insemination was from 5 (for the first inseminated doe) to about 40 min (for the last one).

### Semen processing and artificial insemination

Semen used for AI was obtained from an AI Center, belonging to COGAL SL, where routinely rabbit semen was collected, diluted and stored at 16°C for use within a 24-h period. Ejaculates from 8–12 males (Hyplus PS39, Grimaud Frères, France) were collected using an artificial vagina, pooled and diluted with a commercial extender (MA 24, Ovejero, Leon, Spain) to a standard concentration of  $60 \times 10^6$  spermatozoa·ml<sup>-1</sup>. Only ejaculates with a free-gel volume higher than 0.2 ml and sperm motility (subjective microscopic evaluation) higher than 70% were used. Does were vaginally inseminated using disposable plastic pipettes, receiving a dose of  $30 \times 10^6$  spermatozoa in a volume of 0.5 ml.

### Statistical analysis

Kindling rates were analysed using the Pearson Chi-square test. The litter size at birth (total born, born alive and still born) were analysed using the GLM (General Linear Model) procedure of SPSS 10.0 software (SPSS Inc., Chicago, Illinois, USA), considering the fixed effect of the treatment. The differences between means were tested by the Fisher F test and differences were considered statistically significant at the P<0.05 level.

## RESULTS AND DISCUSSION

The kindling rate was significantly higher ( $P < 0.05$ , Table 1) when the GnRH agonist was added to the seminal dose (91.1% vs. 85.6% respectively for experimental and control groups). This involved an increase in productivity of about 80 kits/100 AI in relation to that obtained when using the control treatment.

**Table 1:** Kindling rates, prolificacy, global productivity (born alive per 100 inseminations) in function of the ovulation-inducing treatment applied. Control: 20 µg of gonadorelin injected intramuscularly; Experimental: 25 µg of [des-Gly10, D-Ala6]–LHRH ethylamide added to the seminal dose

Treatments	Control 20 µg of gonadorelin (i.m.)	Experimental 25 µg of GnRH analogue (i.v.)
Number of AI	270	270
Kindling rates (%)	85.6 <sup>a</sup>	91.1 <sup>b</sup>
Total born	11.1 ± 3.0	11.3 ± 2.7
Born alive	10.3 ± 3.5	10.8 ± 2.8
Still born	0.8 ± 2.1	0.5 ± 1.4
Live-born rabbits/100 AI	949	1029

a,b Different superscript letters indicate significant differences ( $P < 0.05$ ) between means within a row

Buserelin or triptorelin added to seminal doses have been shown (Quintela *et al.*, 2004; Viudes-de-Castro *et al.*, 2007) to be valid methods for inducing ovulation in rabbit does submitted to AI. However, the hormonal doses needed were high and the kindling rates obtained were similar or lower than those for the control group. The present study clearly demonstrated that the GnRH analogue [des-Gly10, D-Ala6]–LHRH ethylamide, added to the seminal dose, was extremely efficient, significantly increasing kindling rates in comparison with the classical use of an intramuscular injection of gonadorelin.

The absorption of GnRH by the vaginal mucosa could be influenced by either the state of mucosa (secretions induced by receptivity state of female), extender composition (organic acid level has positive effects on absorption, Okada *et al.*, 1983), and probably sperm concentration (sperm have an important capacity to incorporate foreign molecules).

In our previous study (Quintela *et al.*, 2004), buserelin was evaluated to determine potential negative effects on the sperm characteristics, and no detrimental effects were found on sperm motility, viability or acrosomal integrity during 24 h of sperm incubation at 16°C. In the present study, the effect of adding the GnRH agonist [des-Gly10, D-Ala6]–LHRH ethylamide to the semen was not evaluated, but it seems evident that significant negative effects were not present. However, it should be interesting to evaluate the sperm characteristics after various periods of incubation in the presence of the agonist.

## CONCLUSIONS

Intravaginal administration of the GnRH agonist [des-Gly10, D-Ala6]–LHRH ethylamide can be successfully used at the dose of 25 µg/doe vehiculated in the seminal dose. This insemination procedure could have some advantages, improving the welfare management of females during insemination by avoiding the intramuscular application of GnRH analogues, increasing the number of females inseminated per operator, and probably reducing the sanitary risk derived from incorrect use of needles. However, more studies are necessary for determining the optimal dose of the hormone, sperm concentration and extender, in relation with the physiological status of rabbit does at the moment of insemination.

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