



Short communication

# Reproductive performance of rabbit does artificially inseminated with semen supplemented with GnRH analogue [des-Gly10, D-Ala6]-LH-RH ethylamide

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## Abstract

The aim of the study was to evaluate, the ability of a GnRH synthetic analogue [des-Gly10, D-Ala6]-LH-RH ethylamide to induce ovulation in rabbit does using intravaginal administration. A total of 138 primiparous lactating does were randomly divided into 4 groups that at the time of insemination received following treatments for ovulation induction: 1  $\mu$ g of buserelin administered intramuscularly (control group); 5  $\mu$ g of [des-Gly10, D-Ala6]-LH-RH ethylamide added to the semen dose (D5 group); 10  $\mu$ g of [des-Gly10, D-Ala6]-LH-RH ethylamide added to the semen dose (D10 group); 15  $\mu$ g of [des-Gly10, D-Ala6]-LH-RH ethylamide added to the semen dose (D15 group); 15  $\mu$ g of [des-Gly10, D-Ala6]-LH-RH ethylamide added to the semen dose (D15 group). Kindling rates were 68.8% in D10 and 66.7% in D15 groups and were comparable to that obtained in the control group (72.2%). The kindling rate in group D5 (29.4%) was significantly lower than those recorded in the other groups. The number of live born kits was not significantly affected by the ovulation induction treatment. The results of this study show that [des-Gly10, D-Ala6]-LH-RH ethylamide added directly into the semen dose can effectively stimulate ovulation in rabbits. The dose of 10  $\mu$ g of [des-Gly10, D-Ala6]-LH-RH ethylamide per doe was sufficient to produce results comparable to those obtained by intramuscular administration of buserelin.

**Key words:** rabbit, ovulation induction, GnRH analogue, insemination

#### Introduction

The physiology of reproduction in the rabbit doe is characterized by induced ovulation. Mating induces a neuro-endocrinological reflex which provokes an LH pulse that leads to ovulation. When using artificial insemination (AI), ovulation has to be induced by artificial methods. The most frequent method used for ovulation induction in rabbits is the intramuscular administration of GnRH or its synthetic analogue. With a view to improving insemination practices, individual ovulation induction by means of subcutaneous or intramuscular treatment of GnRH analogue solution could be substituted by mucosal absorption after

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Group	No. of does	Kindling rate % (no.)	Total kits born Mean ± SD	Live born kits Mean ± SD	
Control	36	72.2 (26) <sup>a</sup>	$9.8 \pm 2.6$	$9.6 \pm 2.7$	
D5	34	29.4 (10) <sup>b</sup>	$10.1 \pm 1.8$	$9.7 \pm 2.1$	
D10	32	68.8 (22) <sup>a</sup>	$9.2 \pm 3.0$	$8.8 \pm 3.1$	
D15	36	66.7 (24) <sup>a</sup>	$9.7 \pm 2.2$	$8.9 \pm 3.1$	

Table 1. Fertility and prolificacy of rabbit does submitted to insemination using different ovulation-induction treatments.

Values in columns with different letters (<sup>a,b</sup>) are significantly different (p<0.05).

supplementation of semen extender with GnRH analogue. This kind of application of the hormone simplifies the AI procedure. Several authors have recently described the addition of different GnRH analogues directly to the semen dose (Viudes de Castro et al. 2007, Rebollar et al. 2012, Gogol et al. 2014, Gogol 2016). Before a routine application of this method of ovulation induction to the rabbit farms it is necessary to determine optimal doses of hormones. Quintela et al. (2009) have revealed that it is possible to induce intravaginal administration ovulation by of [des-Gly10, D-Ala6]-LH-RH ethylamide. Fertility did not significantly differ when ovulation was induced by intramuscular injection of gonadorelin (standard method) or when 25 µg of the GnRH analogue [des-Gly10, D-Ala6]-LH-RH ethylamide was added to the semen dose. Lower doses of this GnRH analogue were not tested. The aim of the present study was to evaluate the effect of different doses of GnRH synthetic analogue [des-Gly10, D-Ala6]-LH-RH ethylamide intravaginally administered on reproductive performance of rabbits.

#### **Materials and Methods**

A total of 138 primiparous lactating commercial hybrid does were used in this study. The does were fed ad libitum with a commercial diet and water was available ad libitum. The bucks used in this study were housed in individual cages with water provided ad libitum whereas feed was restricted to 180 g/day. Semen was collected using an artificial vagina, evaluated for sperm motility and concentration, and diluted with a commercial extender Galap (IMV Technologies, France) to a concentration of 50 x 10<sup>6</sup> spermatozoa/ml and stored at 17°C for 3 hours. Ejaculates with sperm motility higher than 70% were pooled and used for insemination. Does were inseminated using disposable plastic pipettes, receiving a dose of 25 x 10<sup>6</sup> spermatozoa in a volume of 0.5 ml. All the does were inseminated on day 14 after parturition and were suckling 10 kits. To synchronize oestrus a 24-h mother-litter separation was applied. Females were randomly divided into 4 groups and submitted to different treatments for ovulation induction: (1) Control group: 1 µg/doe of buserelin acetate (Receptal, Hoechst A.G., Germany) administered intramuscularly immediately after the insemination; (2) Group D5: 5 µg/doe of [des-Gly10, D-Ala6]-LH-RH ethylamide (Sigma-Aldrich, Saint Louis, MO, USA) administered intravaginally by addition to the semen dose; (3) Group D10 group: 10 µg/doe of [des-Gly10, D-Ala6]-LH-RH ethylamide administered intravaginally by addition to the semen dose; (4) Group D15: 15 µg/doe of [des-Gly10, D-Ala6]-LH-RH ethylamide administered intravaginally by addition to the semen dose. At parturition, the number of total kits born and the number of kits born alive per litter were recorded for subsequent analysis.

A Chi-square test was carried out to analyse the effect of treatment on the kindling rate. Means were compared using t-test and differences were considered significant at p<0.05.

#### **Results and Discussion**

Kindling rates achieved with 10 µg and 15 µg of [des-Gly10, D-Ala6]-LH-RH ethylamide added to the semen dose were comparable to that obtained with conventional insemination technique where ovulation is induced intramuscularly with 1 µg of buserelin (Table 1). The kindling rate in the group stimulated with 5 µg of [des-Gly10, D-Ala6]-LH-RH ethylamide added to the seminal dose was significantly lower than those recorded in the other groups. The number of live born kits was not significantly affected by the ovulation induction treatment. The results of this study confirm the previous observations that [des-Gly10, D-Ala6]-LH-RH ethylamide added directly into the semen dose can effectively stimulate ovulation in rabbits (Quintela et al. 2009). The results do not, however, support the observation that high doses [25 and 30 µg per doe tested in the study of Quintela et al. (2009)] of this GnRH analogue are necessary to obtain normal fertility and prolificacy results. The dose of 10 µg of [des-Gly10, D-Ala6]-LH-RH ethylamide

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per doe was sufficient to produce results comparable to those obtained by intramuscular administration of buserelin.

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