OVULATION INDUCTION IN RABBIT DOES SUBMITTED TO ARTIFICIAL INSEMINATION BY ADDING LECIRELIN TO THE SEMINAL DOSE. PRELIMINARY RESULTS

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ABSTRACT

The aim of this study was to analyze the effect of intravaginal administration of lecirelin on ovulation induction in rabbit does. Eighty pluriparous does (Martini genetic strain) were submitted to AI using a seminal dose of 0.5 ml containing about 10 million sperms. To stimulate ovulation, 4 homogeneous groups were submitted to different treatments: Control Group: 0.2 ml intramuscular administration of lecirelin (Dalmarelin, Fatro®); 0.2 Group: 0.2 ml intravaginal administration of lecirelin; 0.6 Group: 0.6 ml intravaginal administration of lecirelin; 2.0 Group: 2 ml intravaginal administration of lecirelin. In groups receiving an intra-vaginal administration, 25 µg/ml Dalmarelin was diluted in the seminal dose using benzilic alcohol (20 mg/ml) as excipient. Blood samples were collected from all females, to determine LH prior (-60, -30 and 0 minutes) and (30, 60, 90, 120 and 180 minutes) after AI, and progesterone once a week for 4 weeks. After 7 days from AI, 10 does per group were euthanized in order to analyze the ovarian status. The does of control group showed a high LH peak after 30 minutes from AI; whereas intra-vaginal administration of 0.2 and 0.6 ml determined a lower increase of LH blood concentration after 2 hours. The highest dose did not produce any LH or progesterone increase. The ovary status showed a higher number of *corpora lutea* in Control group ($P<0.05$), followed by 0.2 and 0.6 ones, whereas embryos were recorded only in Control and 0.2 groups. The unsuccessful of the other experimental groups could be ascribed to the negative effect of benzilic alcohol on seminal characteristic. Only 30% of 0.2 group does were pregnant and the prolificacy was 8 kits/doe. Compared to the control group, the progesterone concentration in pregnant does showed lower value in 0.2 group. In conclusion, in spite of the obtained results, it will be necessary to test different Dalmarelin formulations (lower volume, different excipient) to recommend the minimal dose to inject intravaginally for inducing ovulation.

Key words: Rabbit doe, ovulation induction, Lecirelin.

INTRODUCTION

In the last years, the productivity of rabbit farms has increased and become more homogeneous through the use of Artificial Insemination (AI), cycled production and very prolific genetic strains (Castellini., 2007). As a consequence of numerous changes on farm management, news operations have been introduced in order to improve the profitability. In rabbit doe the ovulation does not occur spontaneously, but it has to be induced through a neuro-hormonal reflex, which is produced by natural mating (Hafez, 1993). In absence of a male, when AI is applied, ovulation has to be induced by artificial methods. Plasma LH levels start to rise within 3 min after mating and reach a plateau within 15 to 75 min (Jones *et al.*, 1976). Recently, some studies have demonstrated that ovulation can be induced in rabbit females by the vaginal absorption of different GnRH analogous, which are included in the seminal plasma avoiding intramuscular injection (Quintela *et al.*, 2004, Viudes de Castro *et al.*, 2007).

The most diffuse GnRH analogues are buserelin, leuprorelin, goserelin and triptorelin (Dal Bosco *et al.*, 2011); in rabbit doe the gonadoreline (decapeptide) or busereline (nonapeptide) have been shown

to induce ovulation with similar results to those obtained by natural mating (Theau-Clément *et al.*, 1990).

The aim of this study was to analyze the effect of intravaginal administration of lecirelin (GnRH analogues) on ovulation induction in rabbit does.

MATERIALS AND METHODS

Animals and experimental design

The trial was carried out at the experimental farm of the Department of Applied Biology of the University of Perugia (Italy). The environmental temperature ranged from 15 to 28 °C and relative humidity from 60 to 75%. The composition of feed was: crude protein 18.7%, crude fibre 14.7%, fat 4.8% and digestible energy 10.9 MJ/kg (Maertens *et al.*, 1988). Feed and water were provided *ad libitum*. Eighty pluriparous does (Martini genetic) were submitted to AI using an insemination dose of 0.5 ml containing about 10 million sperms (Castellini and Lattaioli, 1999). In order to stimulate ovulation an intra-vaginal inoculation of 25 μg/ml of lecirelin acetate (Dalmarelin - Fatro®) using benzilic alcohol (20 mg/ml) as excipient, was used. In particular does were divided in four homogeneous groups submitted to different treatments:

Control Group: 0.2 ml intra-muscular administration (i.m.) of Dalmarelin (5 μg/doe);

0.2 Group: 0.2 ml intra-vaginal administration of Dalmarelin (5 μg/doe) diluted in the seminal dose;

0.6 Group: 0.6 ml intra-vaginal administration of Dalmarelin (15 μg/doe) diluted in seminal dose;

2 Group: 2.0 ml intra-vaginal administration of Dalmarelin (50 μg/doe) diluted in seminal dose.

The dose insemination varied from 0.5ml (Control Group) to 2.5ml (2 Group).

Ten does per group were euthanized by an intravenous over-dosage of Tanax (Hoechst, Frankfurt, Germany) 7 days after AI, in order to analyze the ovarian status. Two does (one of 0.6 group and another of 2.0 group) died for reason not due to the treatment

To evaluate the effect of Dalmarelin on the spermatozoa vitality, an *in vitro* test was carried out incubating seminal samples of above mentioned treatments at 16 °C for 24 h.

Chemical analyses

In all females blood samples were collected from central ear vein, drawn into non heparinized tubes immediately prior (-60, -30 and 0 minutes) and after (30, 60, 90, 120 and 180 minutes) the insemination. Serum was obtained after centrifugation at 1200 xg for 10 minutes at 4 °C and stored at 32 °C until LH determination by enzyme immuno-assay (EIA, Rebollar *et al.*, 2012).

In addition, blood samples were collected once a week for 4 weeks in ten does from each group, drawn into tubes containing EDTA, immediately centrifuged at 3000xg for 15 min and plasma stored. Progesterone concentrations in plasma samples was determined by radioimmunoassay (RIA), using specific antibody according to the procedure reported by Gobetti *et al*. (1992).

Statistical analysis

Data are given as mean and least significance difference at 5 %. They were processed by GLM procedure of STATA (StataCorp, 2005), based on a linear model evaluating the fixed effect of Dalmarelin dose.

RESULTS AND DISCUSSION

In Figure 1 is reported the evolution of LH concentrations of serum. The does of Control group showed a LH peak 30 minutes after AI; 0.2 and 0.6 groups showed a lower increase of LH after 2 hours. Fifty μg Dalmarelin per doe (2.0 group) did not produce any LH response. Rebollar *et al.* (2012), 60 minutes after AI, recorded an LH peak independently on the inoculation method (1 μg of buserelin i.m./doe *vs.* 10 μg intra-vaginal/0.5 ml fresh semen). Contrarily to our findings, Quintela *et al*. (2004) comparing the effect

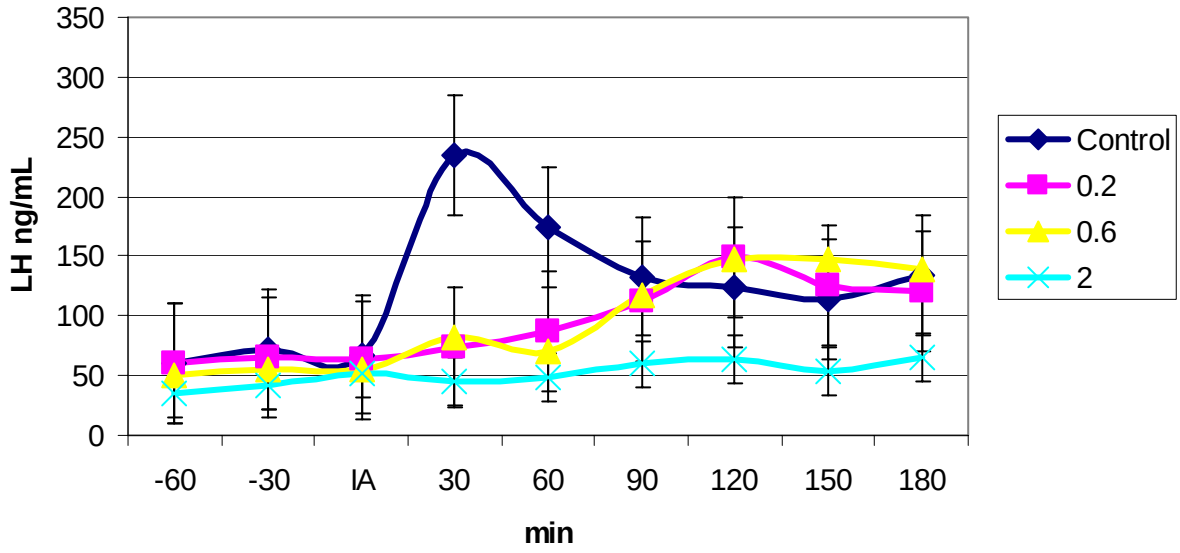

Figure 1. Serum LH concentrations in does before and after ovulation induction with 0.2 ml Dalmarelin i.m. (Control group) or 0.2 ml (0.2 group), 0.6 ml (0.6 group) and 2 ml (2 group) Dalmarelin diluted in the seminal dose, respectively. N=20 per group.

of buserelin administered intravaginally *vs.* i.m. observed an early LH peak in intravaginally administration group followed, 30 min after, by i.m. group. Authors hypothesized that in sexual receptive does, estrogens increase vascularization on the genital tract and increase permeability of the mucosa, which could increase the speed of absorption.

The number of preovulatory follicles and mature follicles was significantly higher in does intravaginally treated (Table 1), while the number of hemorrhagic ones was higher in 0.6 group. As expected, on the basis of LH peaks, ovulation rate was of 100% in Control group followed by 60.0 and 33.3% respectively for 0.2 and 0.6 and 2.0 groups. The number of *corpora lutea* of ovulated does was higher in 0.6 does, but embryos were recovered only in Control and 0.2 groups. Fertility rate reached 80% in Control does and only 30% in the 0.2 ones. The lower number of *corpora lutea* (group 2.0) and the absence of embryos (group 0.6) could be ascribed to the too high volume of seminal dose employed. Indeed, Quintela *et al.* (2004) hypothesized that GnRH could be absorbed only by the vaginal mucose and it does not enter into the uterus. If that was the case, an unknown proportion of analogous may have been lost due to seminal backflow due to high volume inseminated. Thus, the intra-vaginal absorption of GnRH could be negative correlated with the volume of the insemination dose.

Table 1: Ovarian status of does after one week from AI and ovulation induction with 0.2 ml Dalmarelin i.m. (Control group) or 0.2 ml (0.2 group), 0.5 ml (0.6 group) and 2 ml (2.0 group) Dalmarelin diluted in the seminal dose, respectively.

	Does	Preovulatory follicles n	Mature follicles n	Haemorragic follicles n	Ovulation rate % (n)	Corpora lutea[x] n	Embryos[y] n	Fertility rate % (n)
Control	10	6.0[a]	12.3[a]	0.7[a]	100.0[c] (10)	8.3[b]	9.5[c]	80[c] (8)
0.2	10	11.3[b]	26.5[b]	0.7[a]	60.0[b] (6)	8.5[b]	6.5[b]	30[b] (3)
0.6	9	9.7[b]	24.7[b]	1.7[b]	33.3[a] (3)	12.0[c]	0[a]	0[a] (0)
2.0	9	9.0[b]	17.0[ab]	0.3[a]	33.3[a] (3)	2.0[a]	0[a]	0[a] (0)
SED		1.2	2.60	0.2	1.5*	1.5	0.9	25*

In a same column, different letters significantly vary (P<0.05).
[x,y] mean of ovulated and pregnant does, respectively.

Although some does of 0.6 and 2.0 groups ovulated, the absence of embryos and pregnancy could be related to the negative effect of high concentration of benzilic alcohol (excipient of Dalmarelin) on sperm viability, as demonstrated by results of *in vitro* study (sperm mortality of 10, 50, 67 and 72% respectively for Control, 0.2, 0.6 and 2.0 Groups; data not shown).

In Figure 2, it is possible to observe that, in general, the does treated with intra-vaginal Dalmarelin (0.2 group) had a progesterone concentration lower than with an i.m. injection (Control group). Does of 0.2 group which became pregnant (30%) showed a prolificacy of 8 kits/doe (data not shown). From this point of view further research needs to evaluate the eventual differences in progestinic effect of *corpora lutea* of does differently treated to stimulate ovulation.

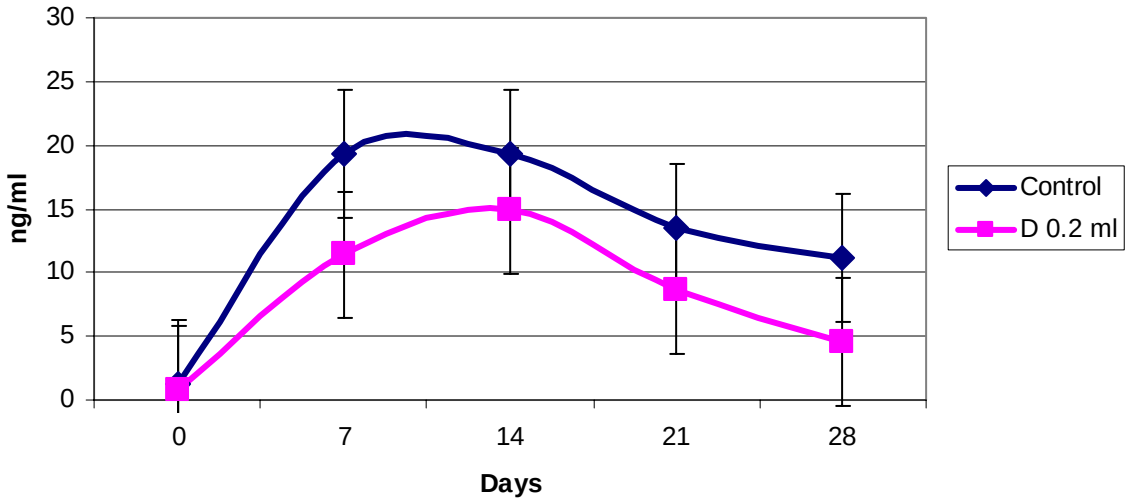

Figure 2: Weekly progesterone concentrations in pregnant does after AI and ovulation induction with 0.2 ml Dalmarelin i.m. (Control group), or 0.2 ml (0.2 group) Dalmarelin diluted in the seminal dose, respectively.

Viudez-de-Castro *et al.* (2007) found that intra-vaginal administration of another analogue (4 and 10 µg Triptorelin /0.5 ml diluted semen) determined a lower ovulation percentage respect to the control group. Vicente *et al.* (2008), analyzing three different genotypes in different rabbit farms, recorded a lower efficacy of buserelin (5 µg/ml dose) on fertility, numbers of kindling and litter size.
On the contrary, Quintela *et al.* (2009) using the GnRH analogue [des-Gly10, D-Ala6]-LHRH ethylamide did not observe differences in kindling rates when ovulation was induced with i.m. gonadorelin (84.5%) or when the molecule was intravaginally administered at the time of AI (93.8 %).

CONCLUSION

In conclusion, despite the not fully satisfying results, this preliminary study, encourage to continue the studies on the ovulation induction in rabbit by the addition of Dalmarelin to the seminal doses providing new perspectives for simplifying the AI technique. Indeed, contrary to findings of other studies, a dose equal to the i.m. has increased the LH and progesterone levels and the low observed fertility seems to be attributable to the negative effect of the excipient on spermatozoa viability. Then, as reported in our previous review (Dal Bosco *et al.*, 2011), additional research is needed to develop some fundamental aspects, and in particular to:
• determine optimal doses that are economically and physiologically effective;
• modify commercial formulations (without harmful excipients for spermatozoa) providing formulations easy to prepare for farmers;
• reduce the insemination volume and sperm concentration for lowering vaginal reabsorption;
• improve the knowledge regarding intraovarian factors that affect ovulation.

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