EFFECT OF LHRH ANALOGUE INCLUDED IN SEMINAL DOSE ON KINDLING RATE AND PROLIFICACY OF RABBITS ARTIFICIALLY INSEMINATED

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ABSTRACT

The objective of our work was to influence positively the induction of ovulation and kindling rate in female rabbits and fertilization capacity of rabbit's spermatozoa by means an intravaginal application of a superanalogue GnRH-Lecirelinum (Supergestran) included in the insemination dose. Experiments were performed on adult female rabbits. Control females were given an intramuscular dose of GnRH-Lecirelinum (Supergestran) (2.5 µg per doe) immediately after the artificial insemination. In experimental females, the insemination doses (I.D.) had different quantities of GnRH-Lecirelinum (Supergestran): 2.5 µg/I.D. (group S2.5), 5.0 µg/I.D. (group S5), 7.5 µg/I.D. (group S7.5) and 15.0 µg/I.D. (group S15), which were incubated 30 minutes at laboratory temperature. Obtained parameters were statistically evaluated by one-way variance analysis. Significance of differences among groups was determined by t-test (LSD) and Duncan's test. Kindling rate (%) in the control group of females was 62.74±13.70. The lowest value of kindling rate was obtained in the group S2.5 (42.99±5.64) and the highest (72.09±2.96) in females of group S7.5. Medium values of kindling rate were reached in females of group S5 (59.97±11.56) and S15 (52.77±3.92). We noticed significant differences in kindling rate of group S2.5 in comparison with those of control, S5 and S7.5 groups only. It would seem that the intravaginal dose in group S2.5 was not sufficient to cause suitable ovulation. Intravaginal application of GnRH-Lecirelinum in the dose 7.5 µg/I.D. influenced positively the induction of ovulation and interactions spermatozoon – zona pellucida of the ovum, with subsequent benefit for the kindling rate in comparison with the control (+9.35%). The average number of kits born alive and kits mortality at birth were not significantly different among the experimental groups of females. Therefore, intravaginal application of GnRH-Lecirelinum in insemination dose did not influence negatively the litter size at birth.

Key words: Rabbits, Artificial insemination, Semen, LHRH, Ovulation.

INTRODUCTION

The work by Chang (1951) was crucial for the study of fertilization. He found out that the rabbit's spermatozoa must spend at least 6 hours in female reproductive tract to be able for fertilization. Fertilization is a complex process, which includes the fertilizing spermatozoon, cumulus oophorus, ZP (zona pellucida) and oolema. Acrosomal reaction plays a fundamental role for penetration of spermatozoon through the mentioned egg membranes. Morales (1998) evaluated the effect of GnRH upon the acrosome reactions, sperm movement characteristics and sperm-zona collisions. Morales *et al*. (2000) tested whether GnRH increases sperm-zona binding in Ca^{2+}-free medium and in the presence of Ca^{2+} channel antagonists.

The objective of the work was to influence positively the fertilization capacity of rabbits' spermatozoa by intravaginal application of a superanalogue of GnRH-Lecirelinum included in the insemination dose and, at the same time, to contribute to do more effective the insemination of rabbits, avoiding the intramuscular application of this analogue.

MATERIALS AND METHODS

Experiments with the intravaginal application of GnRH-Lecirelinum (Supergestran, Czech Republic) in the insemination dose were performed on female rabbits. All females were artificially inseminated (A.I.) by fresh heterosperm semen doses (0.5–1.5 ml per one female). Each female was applied 25 I.U. PMSG i.m. 48 hours before A.I. Every control female was administered intramuscularly 2.5 µg same synthetic GnRH-Lecirelinum as in experimental groups immediately after A.I.

All insemination doses (I.D.) were diluted by insemination extender for rabbit sperm with antibiotic (MiniTüb, Germany) that was diluted in apyrogenic water (MiniTüb, Germany). Concentration of spermatozoa varied from 24.0 to 95.2 x 10^6 per I.D.

In experimental groups, GnRH-Lecirelinum was intravaginally applied together with insemination dose. GnRH-Lecirelinum intravaginally doses were 2.5, 5.0, 7.5 and 15.0 µg per I.D. in S2.5, S5, S7.5 and S15 group, respectively. All of the doses were incubated 30 minutes each at laboratory temperature.

Number of inseminated females, kindled females, live born kits and still-born per litter were observed. The kindling rate was calculated from the ratio of kindled females to the number of mated females.

Obtained parameters were statistically evaluated by one-way variance analysis using the programme SAS 9.1. Significance of differences among groups was determined by t-test (LSD) and Duncan's test.

RESULTS AND DISCUSSION

Results are shown in Table 1. Kindling rate in the control group of females was 62.74±13.70.

Table 1: Reproductive parameters of treated females (mean±SD)

Group	A.I. (n)	Kindling rate (%)	Number of total born kits/litter (n)	Live born kits/litter (n)	Percentage of kits mortality at birth (%)
Control	234	62.74 ±13.70 a	9.03 ±1.71	7.84 ±1.53	11.33 ±17.04
S2.5	148	42.99 ±5.64 b	8.79 ±1.09	8.13 ±0.84	6.99 ±9.03
S5	128	59.97 ±11.55 a	8.82 ±0.42	7.73 ±0.56	12.13 ±7.00
S7.5	61	72.09 ±2.96 a	9.25 ±0.53	7.76 ±0.46	16.01 ±0.24
S15	19	52.77 ±3.92 ab	9.10 ±0.71	7.60 ±0.56	16.47 ±0.27

a,b: $P<0.05$

The lowest value of kindling rate was in group S2.5 and the highest in group S7.5. Medium values of kindling rate were reached in females S5 and S15.

We noticed the greatest variability of kindling rate in the control group (from 38.7 to 80.0%). The lowest variability was observed in group S7.5 (from 70.0 to 74.19%). These findings are in the favor of group S7.5, in which was achieved also higher average kindling rate (+9.35%) in comparison with the control group, even though the statistical difference was not significant. We noticed significant differences in kindling rate only in the group S2.5 with the control, S5 and S7.5 groups. It appears from this that intravaginal dose in group S2.5 (2.5 µg/I.D.) was not sufficient to induce suitable ovulation and interaction spermatozoa-ovum. Intravaginal dose of GnRH-Lecirelinum 5.0 µg/I.D. (S5) showed a tendency to decrease the kindling rate compared with the control group, however, statistically non-significant. Therefore it is possible to state explicitly that the intravaginal application of GnRH-Lecirelinum in a dose 7.5 µg/I.D. (S7.5) influences positively the induction of ovulation and kindling rate. Number of total born kits and percentage of kits mortality at birth were highest in S7.5 and S15 groups. The average number of live born kits was similar among the examined groups of females. It means that intravaginal application of GnRH-Lecirelinum in insemination dose had no negative influence on number of kits in litters.

In our experiments we used by 1 time, 2 times, 3 times and 6 times higher concentrations of GnRH-Lecirelinum in insemination dose compared with intramuscular control (2.5 µg per female). Quintela *et al*. (2004) used GnRH-buserelin in considerable higher concentrations for intravaginal use (10 times up to 20 times) compared with intramuscular control (0.8 µg per female). Differences in kindling rate among treated groups were not statistically significant in their experiments. After intravaginally application of 20-times higher concentration of buserelin they obtained significantly higher prolificacy in experimental females compared with the control (11.7 young and 9.4 kits per litter, respectively).

CONCLUSIONS

Intravaginal application of 7.5 µg of the superanalogue GnRH-Lecirelinum in the insemination dose induced ovulation in female rabbits with subsequent benefit (+9.35 %) for kindling rate without difference with respect to GnRH-Lecirelinum applied via i.m.. Utilization of GnRH-Lecirelinum in insemination dose has no negative influence on litter size at birth.

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