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NON-HORMONAL SUBSTANCES FOR THE INDUCTION OF OVULATION IN RABBIT DOES

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ABSTRACT

In this experiment we studied the effects of different methods for the induction of ovulation on LH concentrations of rabbit does. Eight New Zealand White does were assigned to four groups according to the following ovulatory method employed: natural mating (NM), copper acetate injection (CuAc), GnRH injection and mating with vasectomised buck (VSX). The blood plasma concentrations of LH were 0.27 ± 0.4, 1.05 ± 0.17, 1.18 ± 0.16 and 2.21 ± 0.03 ng/ml for the aforementioned groups during 72 hours after treatment, respectively. During the 8-hours period after either mating or treatment with hormonal or non-hormonal substances there was high correlation between the method for induction of ovulation and LH concentration. Copper acetate injection can be used successfully for the induction of ovulation in rabbits as indicated from the ovulation rate [of the 2 does/ treatment]

INTRODUCTION

In species like the rabbit that do not ovulate spontaneously, the ovulation is normally induced via a neurohormonal reflex started by mating (SAWYER and MARKEE,1950). Injection of GnRH has been used to induce normal ovulation rather than superovulation in rabbit (PAUFLER *et al.*; 1979; LAMB; 1984 and FOOTE and SIMKIN, 1993). Also for the other induced ovulator species, ovulation requires a stimulus to trigger the LH surge responsible for the ovulation process (PAOLICCHI *et al.,* 1999). The ovulatory responses to gonadotropin were studied through plasma concentrations of FSH and LH (CHENG *et al.,* 1999) in rabbit does. Many authors who discovered the role of copper salts to induce ovulation in the female rabbit (FEVOLD *et al*., 1936) postulated that their effect was due to a synergism between copper and the circulating gonadotropins. KISHK (1998) found the same effect on LH concentrations of does which were injected with CuAc to induce ovulation.

The objective of this study was to compare four different methods to induce ovulation: copulation with vasoectomized buck (VSX), intra venous (i.v.) injection of copper acetate (CuAc), i.v. injection of GnRH and natural mating (NM). LH concentrations, shortly after treatment, and ovulation rates were also studied.

MATERIALS AND METHODS

Experimental rabbits:

Eight mature does, two adult bucks and one vasoectomized buck (VSX), all New Zealand White, were used in this experiment. The body weight of does ranged from 3.800 to 4.800 kg, while that of bucks from 3.500 to 4.500 kg. The rabbits were individually caged and raised under normal conditions of temperature (25 ºC), lighting (16 h L), and feeding systems. Also, rabbits had restricted access to rabbit chow, and free access to water.

Catheterizing rabbit for blood collection:
Blood samples were collected according to the method described by MARTIN *et al.,* (1991). This method is designed for collecting blood samples at high frequency rate (every 15 min) by catheterizing the vena Cava, via marginal ear vein.

Determination of LH-hormone by ELISA technique in rabbits:
The LH-assay was carried out using an ELISA procedure according to MOLLER, (1991). The prepared antibody (second antibody, specific for rabbit LH) was used for LH assay in this experiment.

Ovulation induction methods:
Two rabbit does were randomly allocated to each of the following four treatments:

1. In the first group ovulation was induced by copulation with a vasoectomized buck. Vasectomy was done by double legation and removal of a tract of the vas deferens through the abdominal cavity under general anesthesia.
2. In the second group, ovulation was induced by i.v. injection of 2.5 mg/mg CuAc monohydrate (Sigma). For the practical use a 0.7% solution of CuAc was prepared.
3. In the third group, ovulation was induced by i.v. injection of 0.2 ml of GnRH (Fertagyl, Hoechst Veterinar GmbH, Munchen, Germany).
4. In the fourth group, ovulation was induced by natural mating.

In all groups, blood samples were collected according to a specific schedule in which four samples were collected before the mating or artificial insemination (A.I) which were considered as time 0. Thereafter all the does were left for 30 min and then a blood sample was collected every 15 min for four hours and then every 30 min for additional two hours. In some instances, blood samples were collected also daily for 2 or 3 days after the occurrence of ovulation to determine the basal level of LH. The does of all groups were slaughtered 2-4 days after NM or AI and the genital tract removed as soon as possible. Ovarian structures (corpora lutea, follicles larger than 1 mm) were recorded to calculate the ovulation rate. Each uterine horn was flushed with PBS + BSA 0.2% to collect embryos which to evaluate their number and developmental stages by microscopic examination.

Statistical analysis:
Analysis of variance procedure GLM of SAS package (1985) of the repeated measurements model was used to determine the significant differences among different treatments in repeated collected blood samples (45 blood sample for each doe) for plasma LH levels. Also, t-test for paired samples were used to find out significant differences between overall means of different treatments. Correlation coefficient was calculated for mean plasma LH-levels and mean of ovulation rate for all treatments.

RESULTS AND DISCUSSION

The mean values of LH concentrations for each different group are summarized in Table1. Table 2 shows the t-tests analysis of paired samples. The mean level of LH was the highest in VSX group and the lowest in NM group. Also there were no significant differences between CuAc and GnRH group in LH-levels (Table 2). The results of this experiment emphasized that mating is necessary to release sufficient LH within an hour to induce ovulation. In rabbits, beside LH, ovulation requires an intact vascular connection between the pituitary gland and hypothalamus (PAU and SPIES, 1986). Moreover, most of the studies dealing with LH determination showed that that mating evokes a surge of LH secretion lasting for several hours after mating (DUFY *et al.,* 1973; GOODMANN and NEILL, 1976).

As for different groups, VSX group has the highest LH levels compared to the other groups (Table 1 and Fig. 1). The maximal level of LH (7.2 for the first doe and 6.8 ng/ml for the second

one) was reached two h after copulation by VSX buck and then LH decreased gradually to reach 0.8 for the first doe and 0.6 ng/ml after 4.45 h of coitus (Fig 1). After that, LH remained at basal levels (0.05 ng/ml) 5 h after matingfor both does, which were similar to those found before coitus. Where the ovulation rate was 10 for the first doe and 6 for the second, respectively.

Table 1. Mean (± SE) LH plasma concentrations (ng/ml) in the four groups.

Ovulatory induction method	Mean ±SE	Minimum Value	Time After Treatment	Maximum Value	Time After Treatment
VSX Buck	2.21 ± 0.40	0.05	5-hour	7.0	2-hour
GnRH Injection	1.18 ± 0.16	0.05	6.15-hour	3.51	2-hour
CuAc Injection	1.05 ± 0.17	0.05	4.15-hour	3.5	3.3-hour
Natural Mating	0.27 ± 0.03	0.05	2.45-hour	0.70	0.45-hour

Table 2. t-values of means LH plasma in different groups.

Group	Natural Mating	CuAc Injection	GnRH Injection	Mating with VSX buck
Natural Mating		4.72**	4.66**	5.64**
CuAc Injectiom	4.72**		-0.69	3.14**
GnRH Injection	4.66**	-0.69		3.55**
Mating with VSX buck	5.64**	3.14**	3.55**	

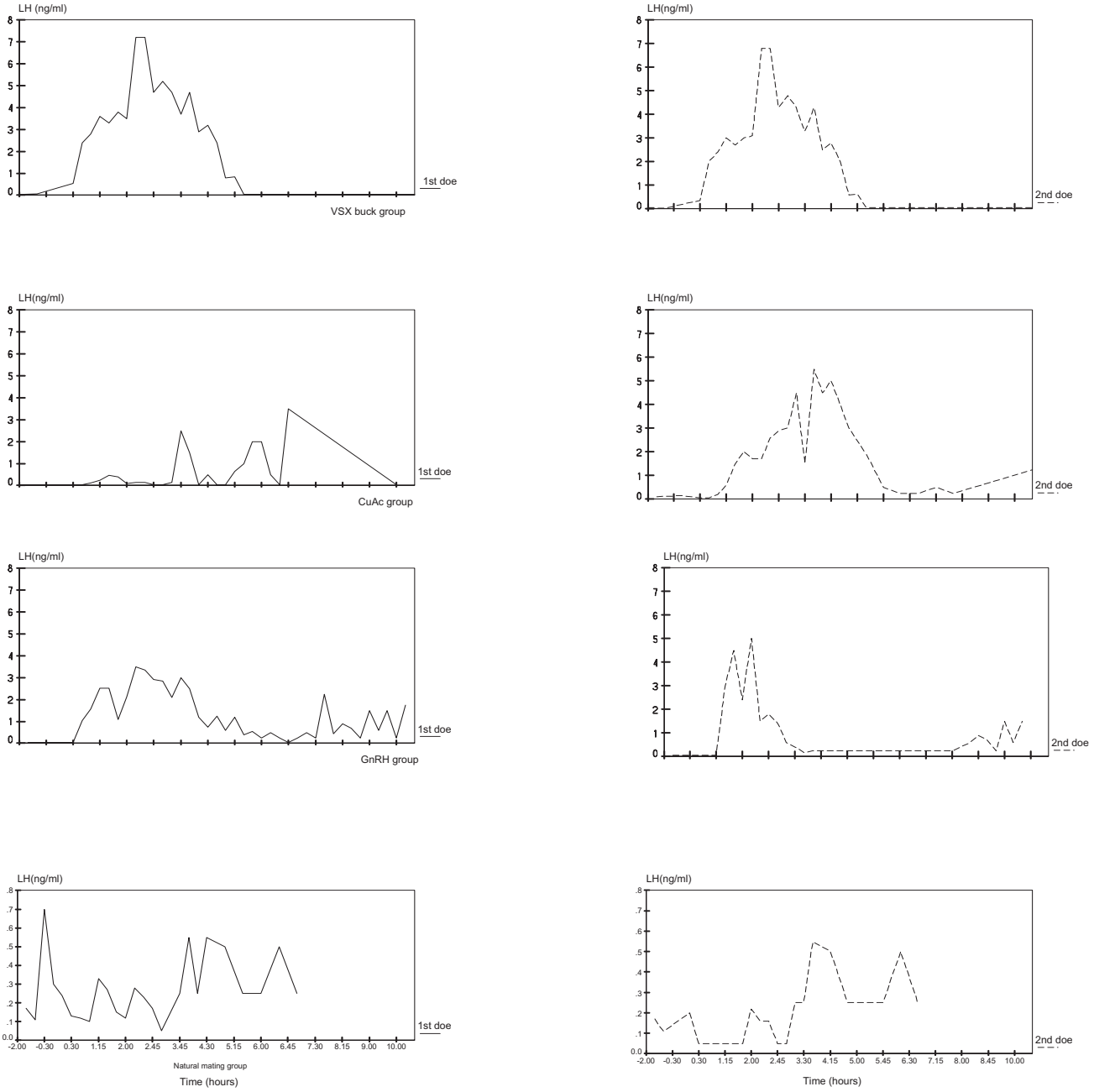
P<0.01

Injection of CuAc caused a gradual increase in LH concentrations which reached their maximal level (3.5 ng/ml) 3.30 h after CuAc administration for the first doe while it was 5.5 ng/ml at 3.30 after coitus as for second doe. These high levels continued for about 4 h (Fig.1) for each dose. After this period, LH-level decreased gradually and reached a basal value 6.30 h after CuAc treatment for both does. Many authors found that CuAc injection induces a surge of gonadotropin secretion that bears both temporal and quantitative similarities with the coitus-induced surge (TSOU *et al.,* 1977). Also, this surge is followed by ovulation (FEVOLD *et al.,* 1936; BROOKS *et al.,* 1940). Also, ovulation rate of CuAc group was 3 for one doe and 1 for the other.

Injection of GnRH produced a gradual increase in LH levels, with a peack 2 h after injection for both does (Fig.1). The high level of LH continued for 3.30 after GnRH injection and then reached a baseline value after 5.30. The low levels of LH-concentration in GnRH group in comparison with VSX group may be explained on the basis of the findings reported by ORSTEAD and SPIES (1987). They found that in the rabbit, a much larger dose of gonadotropin-releasing hormone is required to elicit a significant FSH response that is necessary to stimulate LH release. Ovulation rate average of GnRH group was 1.5.

Ovulation occurred in all groups except for natural mating group. For this reason, the concentration of LH remained at its basal level in all the samples. This finding confirms the necessity of LH in specific concentration to initiate the ovulation process in induced ovulators like the rabbits. Also, KISHK (1998) by comparing natural mating and CuAc injection found that there was no differences in LH concentrations after treatment, but that the ovulation rate was higher in natural mating group than in CuAc. The same trend was noticed in this study as the correlation coefficient between mean plasma LH-levels and mean ovulation rate of different groups was high correlated (r=0.95, P<0.05).

Figure 1. Concentration of LH (ng/ml) following different methods to induce ovulation.



In general this experiment pointed out that hormonal level of LH of VSX group was higher than the other groups and these differences were accompanied by differences in the ovulation rate. So that, there is a direct relationship (r= 0.95) between level of LH shortly (within two hours) after treatment to induce ovulation and ovulation rate for all groups. In addition, the possibility of using CuAc injection for triggering ovulation in female rabbits regardless of their hormonal status and specially for estrogen level has been proved.

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