REPRODUCTIVE PERFORMANCE OF RABBIT DOES BY ADDING LEUPRORELIN IN SEMEN TO INDUCE OVULATION

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ABSTRACT

This trial aimed to evaluate the reproductive performance of rabbit does by adding leuprorelin, a GnRH analogue, in semen to induce ovulation as an alternative method of intramuscular injection. A total of 529 healthy does of line HYLA, including 228 nulliparous strain C does and 301 multiparous strain D does, were divided into five groups randomly and equally as following: (1) L5: adding 5 μg/doe leuprorelin to the seminal dose. (2) L10: adding 10 μg/doe leuprorelin to the seminal dose. (3) L15: adding 15 μg/doe leuprorelin to the seminal dose. (4) PC: intramuscularly injecting with 1 μg/doe buserelin. (5) NC: control group not performed ovulate induction. We collected blood samples from 12 rabbit does each group (6 Strain C, 6 Strain D), respectively, at the time of 0, 1 and 2 hour relative to AI, for determination of the changes of FSH and LH concentrations. Fertility of L10 and L15 were significantly higher than that of NC group (L10: 57.69%; L15: 64.55%; NC: 30.48%), and comparable to PC (PC: 63.46%); while fertility of L5 (49.06%) was significantly lower than that of L15 and PC. Prolificacy parameters were not significantly influenced by the ovulation induction treatments between five groups ($P>0.05$). Due to the induction treatment, significant change of plasma FSH concentration with time both in L15 and PC groups was found ($P<0.05$). Similarly, there were significant changes of plasma LH concentration with time in L5, L15 and PC groups ($P<0.05$). Though the trend of increase of LH concentration with time in L10 group was found, it was not significant ($P>0.05$).These changes, both in FSH and LH levels could explain the better reproductive performances obtained in the L15 and PC groups. Taking together, our study confirmed that adding leuprorelin into the semen could be used to obtain the fertility comparable to those with the general intramuscular injection of buserelin for the ovulation induction in rabbit does. Nevertheless, the injected dose of leuprorelin must be more than 10 μg/doe.

Key words: Leuprorelin; semen; rabbit; artificial insemination; ovulation induction.

INTRODUCTION

Ovulation of rabbit does is induced through a neurohormonal reflex initiated during mating (Julie Bakker *et al.*, 2000). To fulfill the aim of synchronization of ovulation in population of rabbit does, many reagents were applied in rabbit industry. Intramuscular injection of Gonadotropin-releasing hormone (GnRH) synthetic analogues at the time of artificial insemination (AI) was used in the ovulation induction of rabbit does more than 20 years ago (Rodriguez and Ubilla, 1988). Currently, GnRH analogues, buserelin (Receptal, Rebollar *et al.*, 2004; 2008), D-Phe6-GnRH-EA (Ovurelin, Eiben *et al.*, 2007), gonadorelin (Fertagyl, Bonanno *et al.*, 2004), gonadorelina (Inducel GnRH, Rebollar *et al.*, 2006), etc., have been used successfully. In addition, it was found that GnRH and its analogues can be used repeatedly without interfering the immune response of hosts (Kalaba *et al.*, 2011; Adams, 1981).

Intramuscular injection with GnRH occasionally couples with risks of misuse and transmitting diseases if performed by the farmer himself in most small rabbitries, and it is also a time and labor consuming procedure. To avoiding these drawbacks, researchers had showed the possibility of adding

GnRH analogues in rabbit semen to induce ovulation (Quintela *et al.,* 2004; 2009; Vicente *et al.,* 2008; Viudes-de-Castro *et al.,* 2007). Leuprolerin acetate, a nonapeptide chemically defined as 5-Oxo–Pro–His–Trp–Ser–Tyr–D-Leu–Leu–Arg–ProNHEt acetate with a molecular weight of 1209, is a potent agonist of GnRH (Zheng *et al.,* 1999). It has been shown to be 15 fold more active than GnRH (Herbert *et al.,* 2005).

The aim of the present study was therefore: a) to evaluate the efficacy of leuprorelin administered intravaginally in the seminal dose; b) to determine the changes of plasma FSH and LH after treatment with leuprorelin.

MATERIALS AND METHODS

Animals and Environmental conditions

A total of 529 healthy does of line HYLA were used, including 228 nulliparous strain C does (Nulliparous C) and 301 multiparous strain D does (Multiparous D). The nulliparous does were approximately 22 weeks of age and 4 kg of body weight, and the multiparous does had more than 6 parturitions. The trial was executed at the 16th rabbit farm of Kangda Rabbit Co., Ltd. between 23 November 2010 and 8 January 2011. Rabbit does were housed in individual flat-deck cages with a 16L:8D light photoperiod. Does were fed with an *ad libitum* commercial pelleted diet (Kangda NO.576) and free of drinking.

Experimental design

All rabbit does were intramuscularly injected with 25 IU of eCG (HOR-272, Prospec, Israel) 48 h before AI to inseminate. Does were randomly and equally distributed in five experimental groups: (1) L5: adding 5 μg/doe leuprorelin (L3009, sigma, USA) to the seminal dose. (2) L10: adding 10 μg/doe leuprorelin to the seminal dose. (3) L15: adding 15 μg/doe leuprorelin to the seminal dose. (4) PC: intramuscular injection of 1 μg/doe buserelin (B3303, sigma, USA). (5) NC: not performed ovulate induction, adding NaCl to the seminal dose at the same volume as leuprorelin.

Semen processing and artificial insemination

Semen samples were collected from 20 bucks (line HYLA, strain D) using an artificial vagina. After qualification with subjective microscopic evaluation, sperm samples with motility >70%, normal intact acrosome >85% and abnormal sperm <15% were pooled and diluted with a commercial extender (Minitube, Germany) to ensure a minimum concentration of about 50×10^6/ml spermatozoa. All does were inoculated with 0.5 ml of pooled fresh semen in the lordosis position.

Collection of blood samples

To determine the release pattern of follicle stimulating hormone (FSH) and luteinizing hormone (LH), blood samples were collected from 12 rabbit does in each group (6 Strain C, 6 Strain D). Blood samples were collected at three time points, i.e., the time prior to AI and ovulation induction (0 h), 1 h (+1 h) and 2 h (+2h) after AI and GnRH inoculation. Plasma was isolated and stored at -20 °C until analyzed at a later date. All samples were measured using the radioimmunoassay kit (Beijing Purevalley Biotech Co., LTD), following the manufacturer's instruction.

Statistical analysis

Fertility, the total number of kits born, stillborn and live litter weight were recorded at parturition, and average individual weight from each group was calculated. Due to undesired biological reasons (mortality and illness) data were excluded. Fertility was analyzed by the Pearson Chi-square test. Other data were evaluated by mean a general linear model (GLM). The fixed effects were the

ovulation induction procedure (5 levels) physiological status of females (2 levels: nulliparous, multiparous does) and their interaction. Analyses were performed with software SAS version 9.1. Data on the FSH and LH concentrations were analyzed by GLM too. Data were shown as means ± SEM.

RESULTS AND DISCUSSION

Table 1 showed that fertility of three leuprorelin-adding groups (L5, L10, L15) were significantly higher than those of NC group (P<0.05), suggesting that leuprorelin played a positive role in ovulation induction. The fertility of L10 and L15 groups were similar to that of PC group. However, fertility of L5 group was significantly lower compared to PC and of L15 group (P<0.05). To obtain fertility comparable to those obtained with the usual intramuscular injection (1 µg buserelin), at least a 10 µg dose of leuporelin seems to be required. Neither prolificacy, nor live litter weight or individual weight at birth, were significantly influenced by the ovulation method.

Table 1: Reproductive performance of rabbit does in relation with ovulation induced by different treatments.

Group	No. of does	Fertility (%)	Total born (n)	Born alive (n)	Still-born (n)	Live litter weight (g)	Individual Weight(g)
L5	106	49.06 (52)^{b}	8.10±0.44	6.73±0.55	1.37±0.41	467±24	61±2
L10	104	57.69(60)^{ab}	7.70±0.41	6.68±0.51	1.07±0.29	464±24	62±2
L15	110	64.55(71)^{a}	8.10±0.42	6.85±0.52	1.25±0.26	498±20	62±2
PC	104	63.46(66)^{a}	7.78±0.46	6.31±0.59	1.47±0.36	472±25	62±1
NC	105	30.48(32)^{c}	6.84±0.57	5.56±0.69	1.28±0.38	449±28	66±2

L5: 5 µg leuprorelin per doe; L10: 10 µg leuprorelin per doe; L15: 15 µg leuprorelin per doe; NC: negative control; PC: positive control. Within columns, means with different letters are significantly different P<0.05.

As shown in Table 2, Nulliparous does had better reproductive performance than multiparous does, and this was treatment-independent. Nulliparous does were much younger, a higher proportion of them should respond to the estrous synchronization and/or ovulation induction treatments.

Table 2: Reproductive performance of rabbit does in relation with reproductive status.

Group	No. of does	Fertility (%)	Total born (n)	Born alive (n)	Still-born (n)	Live litter weight (g)	Individual Weight (g)
Nulliparous C	228	63.16(144)^{a}	8.62±0.31^{a}	7.14±0.39^{a}	1.48±0.24	495±15^{a}	59±1^{b}
Multiparous D	301	45.51(137)^{b}	6.79±0.23^{b}	5.73±0.31^{b}	1.06±0.18	444±14^{b}	66±1^{a}
Total	529	53.12(281)	7.79±0.20	6.51±0.25	1.28±0.15	472±11	62±1

Within columns, means with different letters are significantly different P<0.01.

In the present study, the efficacy of leuprorelin as a way of vagina absorption to induce ovulation was directed determined by changes of plasma FSH and LH concentrations. Treating with leuprorelin leaded to a significantly increase of plasma FSH concentration with time in L15 and PC groups (Figure 1, a). Treatment with highest leuprorelin dosage (15 µg) or buserelin injection also resulted in the highest fertility, which can be related to the result FSH peak associated with high sexual receptivity (Rodriguez *et al.*, 1989).

The response of plasma LH concentration to leuprorelin (except 10 µg group) and buserelin administration was significant with time (P < 0.05). Results from present study were comparable with previous studies (Rebollar *et al.*, 1997 and 2008 ; Quintela *et al.*, 2004). It also supported that the highest LH concentrations are observed 60–90 min after the administration of exogenous GnRH (Mills and Gerardot, 1984; Rodriguez *et al.*, 1989). Results in the present study showed that efficiency of leuprorelin adding into rabbit semen varied according to different dosages. In the lowest dosage of leuprorelin group (5 µg), the maximum of plasma LH concentration occurred a little later, at the time

point of +2h (Figure 1, b), whereas that of the highest dosage (15 µg) reached the LH peak value at the time point of +1h, and sustained high value to +2h. Although change of LH in leuprorelin 10µg was not significant, trend of that was in accordance with L15 group. As reported in previous studies (Mills and Gerardot, 1984; Rodriguez *et al.*, 1989), plasma LH concentration of i.m. buserelin injection group, reached maximum values 1 hour after, then decreased 1hour later.

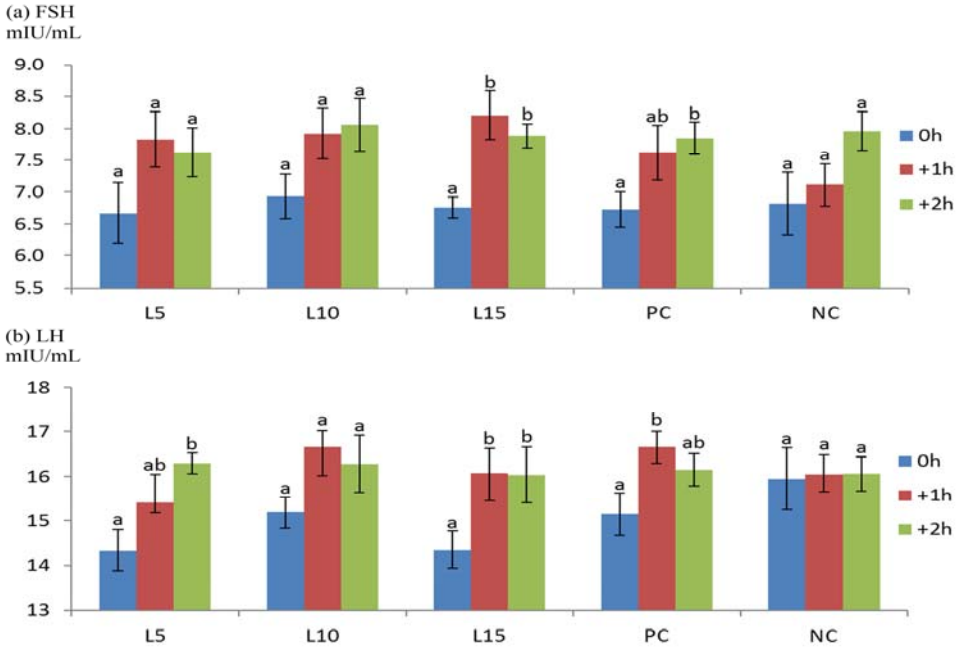

Figure 1: Changes in plasma FSH (1a) and LH (1b) concentrations after different ovulation induction treatments at 0, 1 and 2h after AI. In a same group, means with different letters are significantly different P<0.05.

The present results support the observation of Quintela *et al.* (2004) that a fraction of GnRH analogue may be lost due to seminal backflow, and that high doses of hormone are necessary to obtain normal fertility and prolificacy results (8, 12 and 16 mg per doe in the seminal dose).The changes of LH concentration also support the observation that the intravaginal absorption of the hormone is faster than intramuscular absorption (Quintela *et al.*, 2004).

CONCLUSION

Based on these results and in our experimental conditions, our study confirms that ovulation induction in rabbit does by adding leuprorelin into the semen, is able to lead to the same rabbit productivity at birth to those obtained after a classical intramuscular injection of buserelin. Nevertheless, the injected quantity must be higly increased (1 *vs* 10-15 µg). Further studies will be necessary to define the optimal dose of leuprorelin. In our study, 15µg leuprorelin added into the semen allows to reach the same fertility compared to the classic buserelin administration

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