Reprod. Nutr. Dev. 44 (2004) 79–88

DOI: 10.1051/rnd:2004015

Original article

Ovulation induction in rabbit does submitted to artificial insemination by adding buserelin to the seminal dose

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(Received 1 July 2003; accepted 18 November 2003)

Abstract – This study was aimed at determining if a GnRH analogue, buserelin, could be used for ovulation induction in rabbit does submitted to artificial insemination (AI) by intravaginal administration, by adding the hormone to the seminal dose. In a first experiment, 39 secondiparous experimental does (Hyplus strain PS19, Grimaud Frères, France, of about 30 weeks of age) were divided into 3 groups of 13 does each, which at the moment of AI received the following treatments, respectively: (1) control: an intramuscular injection of buserelin (0.8 µg/doe), (2) 8 µg/doe of buserelin added to the insemination dose, and (3) 16 µg/doe of buserelin added to the insemination dose. The experiment was done using 3 consecutive cycles at 42 day-intervals (n = 39). Four does from each of the 3 groups had blood taken at the fourth cycle for LH determination at 0, 60, 90, 120 and 150 min relative to AI. Kindling rates were 82% (28/34), 56% (29/36) and 85% (33/39), respectively for treatments 1, 2 and 3. In the does of groups 2 and 3, LH peaks were detected 60 min after AI, whereas in the does from group 1, the LH peak was detected 90 min after AI. Prolificacy was not different for the 3 treatments (average litter sizes ranged from 10.4 to 10.8). In a second experiment, 3 buserelin concentrations (8, 12 and 16 µg/doe) were used intravaginally and compared with the control treatment (0.8 µg/doe, via intramuscular). This experiment was done using 100 nulliparous rabbit does (Hyplus strain PS19, Grimaud Frères, France, of about 19 weeks of age) (4 groups of 25 does each) located on a commercial farm, to test if the previous results would be confirmed under field conditions. Kindling rates were no different ($P < 0.05$) for the 4 treatment groups [91.7% (22/24), 79.2% (19/24), 87.0% (20/23) and 87.5% (21/24) respectively for the control, 8, 12 and 16 µg of intravaginal buserelin], however, prolificacy was higher when using the maximal dose of intravaginal buserelin (11.7 vs. 9.4 for the control group). It was concluded that buserelin can be used for ovulation induction in rabbit does when included in the seminal dose, with similar AI results as those obtained when the hormone is administered intramuscularly.

rabbit (*Oryctolagus cuniculus*) / buserelin / intravaginal administration / fertility / prolificacy

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1. INTRODUCTION

The use of artificial insemination (AI) in rabbit farms has become a common practice in European countries. As a consequence, numerous changes on the farm management operations have been introduced. In general, such changes have contributed to improve the life style of the farmer while increasing farm productivity. Still, the current management systems might be further improved, for example, by reducing the administration of exogenous substances [1, 2] or by simplifying the application of those substances that, at least at the moment, cannot be omitted if AI is to be used, such is the case of GnRH that is necessary for ovulation induction.

In the rabbit doe, ovulation does not occur spontaneously, but it has to be induced through a neurohormonal reflex, which is initiated during mating [3]. So, when using AI, in the absence of a male, ovulation has to be induced by artificial methods. The ovulation inducing method most frequently used is an intramuscular injection of GnRH or its synthetic analogues [4–6]. Alternatively, LH or hCG can be used to induce ovulation in rabbit does [7], however, the repeated injection of these hormones can result in failure of the does to ovulate due to antibody formation [8]. Vasectomised males have been used to induce ovulation in does submitted to AI [9, 10], but the method is time-consuming and some females refuse to mate, but ovulate when given LH or GnRH.

So far, the most practical method to induce ovulation in does is the use of GnRH analogues. Gonadoreline or buserelin have been shown [6] to induce ovulation in rabbit does with results similar to those obtained by natural mating. Besides, a major advantage of GnRH is that it can be repeatedly injected without eliciting antibody formation [11]. GnRH analogues are usually applied intramuscularly, however, since they are molecules with a small molecular weight (gonadoreline is a decapeptide and buserelin a nonapeptide), they can also be absorbed subcutaneously or across mucous layers [12, 13].

In most rabbit farms, GnRH administration is usually done by the farmer himself, with a certain risk of misuse, and increasing the time needed for each AI. If GnRH could be used by intravaginal administration, adding the hormone to the seminal dose, with similar results as those obtained by intramuscular injection, it would be beneficial for the farmer. With this method, potential mistakes derived from the hormone not being correctly injected into the muscle would be eliminated, since the hormone could be added in the AI Center when preparing the seminal doses, and it would reduce the time spent by the farmer on the AI of each doe.

The aims of this study were therefore:

(a) to evaluate the AI results, using experimental rabbit does, when buserelin was administered intravaginally by adding it to the seminal dose at two different concentrations (8 and 16 μg per seminal dose), as compared with those obtained when the GnRH analogue was administered intramuscularly;

(b) to investigate plasma LH patterns after buserelin intravaginal or intramuscular administration;

(c) to evaluate the AI results after intravaginal administration of buserelin using rabbit does under field conditions, and testing 3 different concentrations of the hormone (8, 12 and 16 μg per seminal dose), as compared with the results obtained after intramuscular administration of buserelin.

2. MATERIALS AND METHODS

2.1. Animals

2.1.1. Experiment 1

Thirty-nine commercial hybrid does (Hyplus strain PS19, Grimaud Frères,

France) of approximately 30 weeks of age (secondiparous) and between 3.5–4 Kg of body weight were initially used. The does, belonging to an industrial rabbit farm (COGAL SL, Pontevedra, Spain), were housed at the Veterinary Faculty of Lugo for the 6-month experimental period (from November 2001 to April 2002). The animals were randomly divided into 3 groups of 13 does each and located in windowless experimental rooms. A forced ventilation system was used and the inside temperature was maintained between 18 and 22 °C by using an air conditioned-heater system. Light intensity was 70 lux, with an artificial lighting program of 12 h L (light)/12 h D (dark), which was changed to 16 h L/8 h D six days before AI. During the 4 days following AI, light was reduced at 1 h per day, to recover the 12 h L/12 h D photoperiod.

All does were individually housed in flat deck cages (0.3 m^2) communicated through a circular hole with external nests (0.12 m^2), which could be closed by a sliding door.

Pregnant or lactating does were fed ad libitum whereas non-pregnant non-lactating does were restricted to 150 $g \cdot day^{-1}$ of commercial feed except in the period from day 6 before AI to the day of pregnancy diagnosis, during which they were also fed ad libitum. Two types of commercial diets were used: from 21 days post partum to weaning (30–35 days), all does, for practical reasons including non lactating females, were fed with a kits-suitable commercial feed (15.3% CP, 16.5% CF, 1800 Kcal DE), and during all other periods a maternity diet (17.8% CP, 13.3% CF, 2300 Kcal DE) was used.

2.1.2. Experiment 2

One hundred 19 week old nulliparous does of the same strain as that used for experiment 1, located in the original industrial farm (COGAL, SL) together with other 450 does, were randomly chosen for a field trial. The 100 does were randomly divided into 4 groups of 25 does each. The environmental and management conditions on the farm were the same as those described for experiment 1, with the exception that on the farm there were windows and therefore, the does were exposed to natural light. An artificial photoperiod of 12 h L/12 h D (modified around AI as previously specified) was used, but natural light was not prevented from entering in the farm during the light day hours. This experiment was performed during the spring (from May 2003 to June 2003).

The does that died during any of the experiments were not replaced.

2.2. Semen processing and artificial insemination

Experiments 1 and 2

Semen used for AI was obtained from an AI Center, belonging to COGAL SL, where routinely, rabbit semen was collected, diluted and stored at 16 °C for use within a 24 h-period. Ejaculates from 8–12 males (Hyplus PS39, Grimaud Frères, France) were collected using an artificial vagina, pooled and diluted with a commercial extender (MA 24, Ovejero, Leon, Spain) to a standard concentration of 60×10^6 $spermatozoa \cdot mL^{-1}$. Only ejaculates with a free-gel volume higher than 0.2 mL and sperm motility (subjective microscopic evaluation) higher than 70%, were used. Does were vaginally inseminated using disposable plastic pipettes, receiving a dose of 30×10^6 spermatozoa in a volume of 0.5 mL.

2.3. Reproductive management

2.3.1. Experiment 1

All does were inseminated on 3 occasions, at 42 day intervals (i.e. 11 d postpartum), performing a total of 3 AI on the 3 groups. All the does were treated

hormonally to synchronise the oestrus. The hormonal treatment consisted of 20 IU PMSG (Folligon, Intervet, Salamanca, Spain), injected intramuscularly in a volume of 0.4 mL, 48 h before AI.

Controlled suckling was applied to all does from 0 to 10 days post partum, by keeping the nest door closed and only opening it every 24 h, at 12:00 h for 5–10 min, to allow the kits to suck once a day. Controlled suckling before AI is thought to increase the does sexual receptivity at the next AI, since it decreases the antagonism between prolactin and gonadotrophin release. The day of AI (day 11 post partum) suckling was delayed until 17:00 h, 5–10 min before performing the AI. This made a 30 h-mother-litter separation. From day 12 post partum (i.e. 1 day after AI) to weaning (30–35 days post partum) free suckling was allowed by keeping the nest door open. At 11–14 days after AI, all does were diagnosed for pregnancy by transabdominal palpation.

At weaning, the rabbits were sent to an industrial farm to finish their growth, and during that period (i.e. 7–12 days before the next partum) cleaning of the nests and disinfection operations were done in preparation for the next parturition. Parturitions took place mainly on day 30 post AI and in the morning of day 31. For those does not having given birth in the afternoon of day 31, parturition was induced by subcutaneously injecting 2 IU Oxytocin (Hormonipra, Hipra, Girona, Spain). After this treatment (applied to about 5% of the parturitions), labour generally commenced within 5–10 min and it was usually completed within 15 min.

When all does had completed parturition, the number of born alive and dead kits/litter were recorded. Afterwards, the litters were equalled to 10 kits with a homogeneous rabbit size within the litter. Exceeding kits were euthanatised by intraperitoneal injection of Sodium Pentobarbital (Dolethal, Vetoquinol, Madrid, Spain).

2.3.2. Experiment 2

The 4 groups of 25 nulliparous does each, were inseminated on the same day. Only 1 AI was done on each doe ($n = 25$). Synchronisation of oestrous, parturition induction and litter homogenisation were done as described for experiment 1.

2.4. Experimental design

2.4.1. Experiment 1

At the moment of AI, the 3 groups of 13 does each, received different hormonal treatments for ovulation induction:

(1) Control group: 0.8 μg/doe of buserelin (Receptal®: 4 $\mu g \cdot mL^{-1}$, Intervet, Salamanca, Spain), injected (0.2 mL) intramuscularly.

(2) 8 μg/doe of buserelin (Suprefact®: 1 $mg \cdot mL^{-1}$ buserelin in an aqueous solution containing benzyl alcohol, sodium phosphate $2H_2O$, sodium chloride and sodium hydroxide; Aventis Pharma, SA, France), administered intravaginally by adding the hormone (8 μL) to the seminal dose.

(3) 16 μg/doe of buserelin (Suprefact®), administered intravaginally by adding the hormone (16 μL) to the seminal dose.

The buserelin preparation Suprefact® is indicated for use in humans. It was used experimentally instead of Receptal® due to its higher concentration, which allowed us to add a small volume of the product to the seminal dose.

Four does randomly chosen from each of the 3 groups received a fourth AI. Just before the AI (hour 0), a blood sample was collected from all does. After AI, the blood samples were collected at 60, 90, 120 and 150 min from the 12 does, to determine plasma LH.

Preliminary testing of the buserelin effect added to the seminal dose

Before starting the present experiment, a pilot study was done in order to detect a potential harmful effect on seminal characteristics as a consequence of adding buserelin. Ejaculates were collected from 3 males on 3 occasions (9 ejaculates) and diluted with MA 24 at a sperm concentration of 60×10^6 spermatozoa·mL^{-1}, as described previously. Each semen sample was divided into two equal volumes, one was a control sample and the other one was added 32 µg·mL^{-1} of buserelin (Suprefact®). The two seminal samples were incubated at 16 °C for 24 h. After 0, 8 and 24 h of incubation the following sperm quality parameters were assessed: the percentage of motile spermatozoa, by subjective microscopic examination, the percentage of live and abnormal spermatozoa, by using eosin-nigrosin staining, and the percentage of spermatozoa with intact acrosomes, as determined by Spermac® staining. The addition of buserelin to semen was not found to have negative effects on any of the sperm quality parameters evaluated.

2.4.2. Experiment 2

At the moment of AI, the 4 groups of 25 does each, received the following hormonal treatments for ovulation induction:

(1) Control: 0.8 µg/doe of buserelin (Receptal®) injected intramuscularly (0.2 mL).

(2) 8 µg/doe of buserelin (Sigma, St. Louis, MO, USA). The powder was diluted in saline solution (1 mg·mL^{-1}) just before use and it was administered intravaginally adding 8 µL of the solution to the seminal dose.

(3) 12 µg/doe of buserelin (Sigma), administered intravaginally (12 µL) as described for group 2.

(4) 16 µg/doe of buserelin (Sigma), administered intravaginally (16 µL) as described for group 2.

In this experiment, raw buserelin was used instead of Suprefact® to avoid including other excipients (1.0% benzyl alcohol in water) except saline solution.

2.5. Collection of blood samples

Blood samples were collected to determine the plasma LH pattern release in response to exogenous buserelin administered at the time of AI. Samples were collected from the margin ear vein into EDTA tubes and immediately centrifuged at 1000 *g* × 10 min. Plasma was stored at –20 °C until analysis.

2.6. Hormone analysis

Rabbit LH (RbLH) was analysed by using an Enzyme Immunoassay (EIA) procedure described previously [14]. A purified mouse monoclonal antiserum (518B7) raised against the β subunit of bovine LH was used as the primary capture antibody. The enzyme-labelled second antibody was anti-rabbit IgG-peroxidase from the goat (A05045), and the substrate was 3,3',5,5'-tetramethyl benzidine dihydrochloride (TBM). Conversion of the substrate by the enzyme was measured in an automatic plate reader (Biotek Instruments Inc., Winooski, VT, USA) at 450 nm. The smallest amounts of RbLH that could be distinguished from zero concentration were 16.1 ± 1.42 pg·mL^{-1}. The precision of the EIA was assessed in selected pooled serum samples. Average intra-assay CV for pools of high, medium and low LH concentrations in serum were 5.4, 7.1, and 9.8%, respectively, whereas the average inter-assay CV for these same pools were 6.4, 8.8, and 12.1%, respectively.

The accuracy of the EIA was determined by measuring the recovery rates of known amounts of RbLH (0, 1, 5, 10, 25 ng·mL^{-1}) which had been added to the different pools of serum containing no LH. The recovery rates averaged (mean ± SE) 96.54 ± 1.87 for the higher values

Table I. Kindling rates, prolificacy, mortality and global productivity (the number of rabbits born alive per 100 inseminated does) in function of the ovulation-inducing treatment applied. Control treatment: 0.8 µg of buserelin (Receptal®) injected intramuscularly; treatments 2 and 3: respectively, 8 and 16 µg of buserelin (Suprefact®) added to the seminal dose.

Treatments	1 (control) (n = 34)	2 (n = 36)	3 (n = 39)
Kindling rates (N or %)	28/34 (82.4%)[a]	20/36 (55.6%)[b]	33/39 (84.6%)[a]
Prolificacy	10.8 ± 3.1	10.4 ± 2.2	10.5 ± 2.6
Mortality/litter at birth	0.2 ± 0.4	0.7 ± 2.0	0.4 ± 0.6
Live-born rabbits/100 AI	870	539	848

[a,b] Different superscript letters indicate significant differences ($P < 0.05$) between means within a row.

(10.0–25.0 ng·mL^{-1}) and 94.58 ± 4.65 for the lower concentrations of RbLH (0.0–5.0 ng·mL^{-1}).

2.7. Statistical analysis

Data on kindling rates were analysed by the Pearson Chi-square test. The differences were considered statistically significant at the $P < 0.05$ level. Data on the total number (prolificacy) and number of young born dead (mortality), were analysed using the GLM (General Linear Model) procedure of SPSS 10.0 software (SPSS Inc., Chicago, Illinois, USA), considering the effects of the treatments. The differences between means were tested by the Fisher F test. Data on the LH concentrations were not analysed statistically due to the low number of animals used.

3. RESULTS

3.1. Experiment 1

3.1.1. Kindling rates, prolificacy and mortality

The ovulation-inducing treatment had a significant ($P < 0.05$) influence on the kindling rates. The does in groups 1 (control) and 3 had higher kindling rates compared with the does in group 2 (82.4% and 84.6% vs. 55.6%, respectively, Tab. I). Prolificacy and mortality were not significantly influenced by the ovulation-inducing treatments.

The 3 groups of does used for LH determination at a fourth AI were all pregnant except one doe belonging to the control group.

3.1.2. LH

In the two groups of does that received intravaginal administration of buserelin (groups 2 and 3), LH peak concentrations were detected in blood samples collected 60 min after AI (36.6 ± 9.3 and 37.8 ± 6.2 ng·mL^{-1}, respectively for groups 2 and 3), whereas in the control group the LH peak (35.1 ± 6.1 ng·mL^{-1}) was detected in blood samples collected 90 min after AI (Fig. 1). At 150 min after AI, the 3 groups of does had basal LH concentrations (range from 4.0 ± 1.4 to 5.7 ± 3.6 ng·mL^{-1}), as compared with those observed before buserelin administration (range from 3.4 ± 1.9 to 4.9 ± 1.0 ng·mL^{-1}).

3.2. Experiment 2

In the field trial, the kindling rates were not statistically different for the 4 hormonal treatments used. However, a tendency

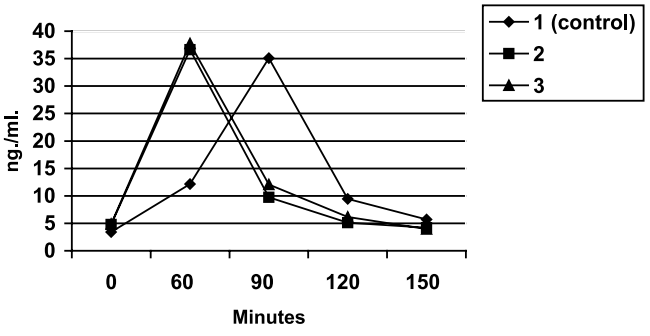

Figure 1. Plasma LH concentrations obtained before (0 min) and after AI (1 h, and then at 30 min-intervals) when using 3 ovulation-inducing treatments. 1 (control treatment): 0.8 µg of buserelin (Receptal®) injected intramuscularly; treatments 2 and 3: respectively, 8 and 16 µg of buserelin (Suprefact®) added to the seminal dose.

was seen for the kindling rate to be lower when the lowest dose of buserelin was added to the seminal dose (Tab. II). Prolificacy was significantly higher ($P < 0.05$) when the highest dose of buserelin was added to the seminal dose (11.7 vs. 9.4, 9.9 and 9.7 respectively for groups 1, 2 and 3), and this involved an increase in productivity of about 140 kits /100 AI in relation to that obtained when using the control treatment.

4. DISCUSSION

The present study clearly demonstrates that the GnRH analogue buserelin, can be added to the seminal dose, as a method to induce ovulation in rabbit does submitted to AI. However, to obtain fertility rates comparable to those obtained with the usual intramuscular injection, a buserelin dose at least 15 fold higher seems to be required. The lowest dose of buserelin used in this study, 10 fold higher than the control one, produced significantly lower fertility rates than the control treatment when applied to multiparous does, whereas the difference was not significant when used in nulliparous does.

Nulliparous does had better fertility than multiparous does, independently of the treatment used, probably because they were not lactating, and a higher proportion of females responded to the estrous synchronisation and/or ovulation induction treatments. A hormonal antagonism between prolactin and gonadotrophin release has been demonstrated in lactating rabbits [2, 15]. In multiparous does, prolificacy did not vary with the different treatments, whereas in nulliparous females, the highest dose of intravaginal buserelin increased prolificacy in about 1.7 kits/litter compared with the control treatment, which made an increase in productivity of about 140 kits per 100 inseminated does. These results did not agree with those of Perrier et al. [16], who found that nulliparous does had lower fertility and prolificacy than multiparous females. Nevertheless, nulliparous does in this study were about 19 weeks old, whereas in that of Perrier et al. [16] they were 16 weeks old. Besides, it has been suggested [17] that genetic factors might influence buserelin sensitivity.

Administration of buserelin resulted in an LH response in the three groups of does evaluated. The serum concentrations of LH obtained were comparable with those

Table II. Kindling rates, prolificacy, mortality and global productivity (the number of rabbits born alive per 100 inseminated does) in function of the ovulation-inducing treatment applied. 1 (control treatment): 0.8 μg of buserelin (Receptal®) injected intramuscularly; treatments 2, 3 and 4: respectively, 8, 12 and 16 μg of buserelin (Sigma) added to the seminal dose.

Treatments	1 (control) (n = 24)	2 (n = 24)	3 (n = 23)	4 (n = 24)
Kindling rates (N and %)	22/24 (91.7%)	19/24 (79.2%)	20/23 (87.0%)	21/24 (87.5%)
Prolificacy	9.4 ± 2.1[a]	9.9 ± 3.1[ab]	9.7 ± 2.1[a]	11.7 ± 2.0[b]
Mortality/litter at birth	1.1 ± 2.3	1.5 ± 3.0	1.5 ± 2.4	1.4 ± 2.7
Live-born rabbits/100 AI (Nº)	767	662	713	907

[a,b] Different superscript letters indicate significant differences ($P < 0.05$) between means within a row.

observed by Rebollar et al. [18] and Ubilla et al. [19]. In rabbit does, the highest LH and FSH concentrations have been found [20, 21] to occur 60–90 min after exogenous administration of GnRH, which was confirmed in the present study.

Intravaginal administration of buserelin, at the two concentrations tested, induced an earlier LH peak than the intramuscular injection (60 vs. 90 min). Although the number of animals used for LH determination was small, these preliminary results may suggest that the absorption of the hormone is faster intravaginally than intramuscularly. During estrous, the female genital tract is under the effects of estrogens secreted by the mature Graafian follicles [3]. Estrogens are known to increase vascularisation of the genital tract and to cause an increased permeability of the blood vessel walls [3], therefore, facilitating the absorption of substances through the genital tract mucose. Nevertheless, whether exogenous GnRH absorption is faster when administered intravaginally than intramuscularly has still to be investigated.

The absorption of buserelin might be faster by intravaginal than by intramuscular administration, and to reach effective blood concentrations, much higher doses of buserelin were needed when using intravaginal administration. An important fraction of the hormone vehiculated in the inseminated dose is likely to have been lost or not absorbed. Some biochemical constituents of seminal plasma, such as prostaglandins, together with the mechanical stimulus of natural mating, both are thought to stimulate the smooth muscle activity of the female reproductive tract and thereby contribute to the distribution of semen and spermatozoa within the tract [22, 23]. When using AI, however, seminal plasma was not found to enter the uterus and oviducts of rabbits [24], while the spermatozoa actively migrated through the cervix. This fact might indicate that, in the present study, the hormone was absorbed only through the vaginal mucose and it did not enter the uterus. If that was the case, an unknown proportion of buserelin may have been lost due to seminal backflow. Perhaps the amount of buserelin absorbed intravaginally could be increased by reducing the volume of the insemination dose, but probably the effective dose will still have to be higher than that used by intramuscular injection.

The buserelin preparations used in this study, with the exception of Receptal®, are not licensed for use in animals. In the case that a buserelin product indicated for intravaginal administration in rabbits is approved and marketed, its cost would have to be

comparable to that of GnRH preparations commonly used on rabbit farms. Alternatively, more potent agonists of GnRH, such as Nafarelin or Deslorelin, deserve further investigation as potential methods for ovulation induction by intravaginal administration in rabbits.

In conclusion, the results of the present study are promising regarding the possibility of using GnRH analogues, vehiculated in the seminal dose, as ovulation inducting treatments for rabbit does bred by AI. More research is, however, needed in order to determine the optimal GnRH analogue and the optimal dose that makes it economically efficient and physiologically effective.

ACKNOWLEDGEMENTS

The authors thank Drs. MJ Illera and JC Illera for performing the LH assays in their Lab. (Department of Physiology, Faculty of Veterinary Medicine, Universidad Complutense, Madrid, Spain).

The study was supported by Xunta de Galicia (Plan Gallego de Investigación y Desarrollo Tecnológico, Proyecto Ref. 2000/CG452) and by Conejos Gallegos, COGAL S.L., Rodeiro, Pontevedra, Spain.

REFERENCES

[1] Quintela L, Peña A, Barrio M, Vega MD, Díaz R, Maseda F, García P. Reproductive performance of multiparous rabbit lactating does: effect of lighting programs and PMSG use. Reprod Nutr Dev 2001, 41: 247–257.

[2] Theau-Clément M. Advances in biostimulation methods applied to rabbit reproduction. 7th World Rabbit Congress, Valencia, Section Reproduction and Reproductive physiology, Main paper C, 2000.

[3] Hafez ESE. Reproducción, hormonas y factores de crecimiento. In: Reproducción e Inseminación Artificial en Animales. 6th edn. Hafez ESE (Ed), Mexico, DF: Interamericana-McGraw-Hill, 1993.

[4] Battaglini M, Constantini F, Baldissera CN, Castrovilli CF. Induction of ovulation and artificial insemination in the rabbit. Coniglicoltura 1982, 19: 45–54.

[5] Michelmann HW, Paufler SAI. Results following the use of LH-releasing hormone in rabbits. Zuchthygiene 1973, 8: 178.

[6] Theau-Clément M, Bolet G, Roustan A, Mercier P. Comparaison de différents modes d'induction de l'ovulation chez les lapines multipares en relation avec leur stade physiologique et la réceptivité au moment de la mise à la reproduction. 5es Journées de la Recherche Cunicole, 12–13 Décembre, Paris, 1990, Tome I, Communication 6.

[7] Foote RH, Hafs HD, Staples RE, Gregoire AT, Bratton RW. Ovulation rates and litter sizes in sexually receptive and nonreceptive artificially inseminated rabbits given varying dosages of luteinizing hormone. J Reprod Fertil 1963, 5: 59–66.

[8] Adams CE. Induction of ovulation and AI techniques in the rabbit. Vet Rec 1972, 91: 194–197.

[9] Khalifa RM, El-Alamy MA, Beshir MA. Vasectomized buck gave better reproductive results in artificial insemination techniques in rabbit than GnRH or HCG. 7th World Rabbit Congress, Valencia, Spain 2000.

[10] Khalifa RM. Mating frequency and artificial insemination in rabbit using vasectomized bucks. World Rabbit Sci 1994, 2: 1–5.

[11] Adams CE. Artificial insemination in the rabbit: The technique and application to practice. J Appl Rabbit Res 1981, 4: 10–13.

[12] Camier B, Gagneur O, Arlot S, Thépot F, Vitse M. Preliminary study of the use of intranasal Buserelin in the long-term protocol of ovarian stimulation with the view toward fertilization in vitro. Rev Fr Gynecol Obstet 1989, 84: 659–661.

[13] Donnez J, Nisolle-Pochet M, Cleckx-Braun F, Sandow J, Casanas-Roux F. Administration of nasal Buserelin as compared with subcutaneous Buserelin implant for endometriosis. Fertil Steril 1989, 52: 27–30.

[14] Illera JC, Munro CJ, Silván G, Bondurant RH, Illera M. Development of a simple, rapid sandwich enzyme immunoassay for the measurement of serum rat LH. J Physiol Biochem 1996, 52: 95–102.

[15] Theau-Clément M, Roustan A. A study on relationships between receptivity and lactation in the doe, and their influence on reproductive performances. J Appl Rabbit Res 1992, 15: 412–421.

[16] Perrier G, Theau-Clément M, Jouanno M, Drouet JP. Reduction of the GnRH dose and

inseminated rabbit doe reproductive performance. 7th World Rabbit Congress, Valencia, Spain 2000.

[17] Foote RH, Simkin EM. Use of Gonadotropic releasing hormone for ovulating the rabbit model. Lab Anim Sci 1993, 43: 383–385.

[18] Rebollar PG, Alvariño JMR, Illera JC, Silván G. Effect of gonadoreline and naloxone on induction of ovulation and plasma LH in rabbit. J Physiol Biochem 1997, 53: 205–210.

[19] Ubilla E, Rebollar PG, Pazo D, Esquifino AI, Alvariño JMR. Pituitary and ovarian response to transient doe-litter separation in nursing rabbits. J Reprod Fertil 2000, 118: 361–366.

[20] Mills T, Gerardot RJ. Dissociation of copulation from ovulation in pregnant rabbits. Biol Reprod 1984, 30: 1243–1252.

[21] Rodriguez JM, Agrasal C, Esquifino A. Influence of sexual receptivity on LH, FSH and prolactin release after GnRH administration in female rabbits. Anim Reprod Sci 1989, 20: 57–65.

[22] Harper MJK. Gamete and zygote transport. In: Knobil E, Neill JD (Eds), The Physiology of Reproduction, 2nd ed, Raven Press, New York, 1994, p 123–187.

[23] Overstreet JW, Katz DF. Interaction between the female reproductive tract and spermatozoa. In: Gagnon C (Ed), Controls of Sperm Motility: Biological and Clinical Aspects, CRC Press, Boca Raton, Florida, 1990.

[24] Asch RH, Balmaceda J, Pauerstein CJ. Failure of seminal plasma to enter the uterus and oviducts of rabbit following artificial insemination. Fertil Steril 1977, 28: 671–673.